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ABSTRACT

APPLICATION OF SOLID PHASE MICROEXTRACTION COUPLED WITH GAS CHROMATOGRAPHY/MASS SPECTROMETRY AS A RAPID METHOD FOR FIELD SAMPLING AND ANALYSIS OF CHEMICAL WARFARE AGENTS AND TOXIC INDUSTRIAL CHEMICALS

by

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Uniformed Services University of the Health Sciences, 2003

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The first question that must be answered prior to substantive quantitative exposure monitoring, regardless of the sampling and analysis method employed, is: What chemicals are present? In order to answer this question rapidly, there is increasing demand for field analysis of volatile and semi-volatile organic compounds with instrumentation that provides definitive identification. The military's interest in this capability stems from Presidential Review Directive 5 and other Department of Defense implementing instructions that have established the requirement

for developing better means for operational exposure assessments and documentation of troop exposures during deployments.

Numerous methods have been developed for field analysis of organic compounds. However, these methods may have limitations such as lack of sensitivity, high false positive identification rates and provide only screening capabilities. Laboratory analysis, often with complex sample preparation requirements, is still required for confirmation of a chemical's identification.

The objective of this work was to evaluate the use of solid phase microextraction (SPME) coupled to gas chromatography/mass spectrometry (GC/MS) as a rapid method for field sampling and analysis to answer the important question of "What chemicals are present?" and other related questions that may arise.

To reach this objective, SPME sampling followed by analysis with a field portable GC/MS system was evaluated. The qualitative abilities of SPME-GC/MS were evaluated in an industrial workplace containing air contaminants from a poorly characterized paint and in an unplanned, emergency response situation. The qualitative abilities were further examined through development of field sampling and analysis methods to detect the chemical warfare agent (CWA) VX as a contaminant on clothing and soil. The quantitative potential for SPME-GC/MS was

examined through the development of a rapid method for estimating the airborne concentration of the CWA sarin.

This work demonstrated the robust nature and flexibility of the SPME-GC/MS system to rapidly detect, identify, and quantitate organic chemicals of widely varying volatility in the field while providing high quality data. This ability gives rise to wide application in industrial, emergency response, homeland security and military deployment operations for characterization of potential health, safety, and security hazards.

APPLICATION OF SOLID PHASE MICROEXTRACTION COUPLED WITH GAS CHROMATOGRAPHY/MASS SPECTROMETRY AS A RAPID METHOD FOR FIELD SAMPLING AND ANALYSIS OF CHEMICAL WARFARE AGENTS AND TOXIC INDUSTRIAL CHEMICALS

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Department of Preventive Medicine and Biometrics,

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of

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DEDICATION

I dedicate this dissertation to my family who has allowed me to be a full-time student and part-time husband and father during this endeavor. Georgia, you have supported me through an undergraduate, masters and now a doctoral program. You have endured every step of my Navy career. You truly know the meaning of sacrifice. You give me your never-ending love. You are my life. Nathan, you bring music to my ears. Eli, you bring laughter to my lips. Abby, you bring beauty to my eyes. You all bring joy to my heart. For these I am eternally grateful to God.

I would be amiss to not extend this dedication to my parents, Virgil and Betty Hook, and my wife's parents, Elzie and Ruth Anne Wymer. Your love has been an inspiration to us.

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CHAPTER 1

INTRODUCTION

For legal and moral reasons, occupational and environmental health professionals are required to identify, monitor, and document worker exposures to health hazards in their location of employment. In the civilian community, the Occupational Safety and Health Administration (OSHA) has established the requirement for employers to control, monitor and document worker exposures to chemical hazards in their workcenters. This requirement has been reasonably well met in industrial workcenters where exposures are constant and have been thoroughly characterized. Meeting this requirement becomes more difficult when material safety data sheets (MSDS) and other related documents do not accurately provide information concerning the chemicals present in the materials being used or when unexpected worker exposures could occur to extremely hazardous chemicals as in the case with emergency response personnel. Unfortunately, with the ever-present potential for acts of terrorism, it is more important than ever that emergency response personnel have the capability for on-scene chemical detection and identification to ensure they are adequately protected on the scene and that those affected receive appropriate treatment.

In the operational environments, like those typical of U.S. military deployments, chemical exposures are often unknown, uncharacterized and uncontrolled for the most part and could range from exposure to toxic industrial chemicals to a variety of chemical warfare agents. Unlike industry, exposure of deployed military personnel is not limited to a 40 or 50 hour work week but can be 24 hours per day for weeks or even months at a time. Obviously, typical sources of information like the MSDS are not available for military operations outside of routine equipment maintenance. Monitoring personnel must rely on intelligence information (e.g. from the Armed Forces Medical Intelligence Center (AFMIC)) or personnel in country to guide quantitative exposure monitoring for deployed personnel. At best, this provides general guidance and often leaves unanswered the most important question that must be asked prior to substantive quantitative exposure monitoring which is: What chemicals are present?

In large part to answer this question, there is growing demand in the civilian and military communities for rapid, laboratory quality, field analysis of volatile and semi-volatile organic compounds [1,2,3,4]. Current field detection instrumentation provides a chemical screening capability, which for the most part does not provide the quality of data needed to provide confirmation of a chemical's identification. Confirmatory analysis typically requires a level of analytical instrumentation not common

outside of a laboratory. In addition, introduction of a sample to these laboratory grade analytical instruments can require extensive preparation steps that do not easily lend themselves to non-laboratory environments. The requirement to send samples for confirmatory analysis to a fixed laboratory facility only serves to delay the receipt of data that can be time critical.

Research Question

Gas chromatography/mass spectrometry (GC/MS) has long been recognized as the "gold standard" for identification of important classes of unknown chemicals. Advances have been made in GC/MS technology making GC/MS instruments smaller, more sensitive and more durable. Advances in sampling and sample preparation have also been made, including development of solid phase microextraction (SPME), a method that can simplify and shorten sampling and sample preparation requirements in many cases. In combination, these advances have the potential to provide an improved fieldable sampling and analysis system. The question to be addressed by this research is: Can solid phase microextraction coupled with gas chromatography/mass spectrometry provide a rapid method for field sampling and analysis of chemical warfare agents and toxic industrial chemicals?

Research Objectives

In order to establish the usefulness of gas-phase sampling with SPME combined with field GC/MS analysis for a wide range of chemicals and sampling environments, methods were developed for detection of three types of materials: a paint primer widely used on vessels throughout the U.S. Navy, O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate (VX) as a clothing and soil contaminant, and airborne isopropyl methylphosphonofluoridate (sarin, or GB). The specific objectives were to (1) qualitatively identify components of Formula 150 paint primer not listed on the MSDS while in a complex industrial environment, (2) develop a method to qualitatively detect VX on soil, (3) develop a method to qualitatively detect VX on clothing and, (4) develop a method to qualitatively and quantitatively detect airborne sarin. A primary consideration in development of these methods was that their usefulness should be demonstrated using field-portable GC/MS instrumentation.

Formula 150 primer is a two-part epoxy widely used by the U.S. Navy. It was selected for study in order to examine the ability of the SPME-GC/MS system to sample and analyze airborne chemical hazards in a complex environment that has been well characterized by industrial hygienist for a number of years. Only *n*-butanol was directly identified in the primer's material safety data sheet supplied by the manufacturer. Other ingredients remain individually unnamed and are simply titled #100

solvent. Exposure monitoring for this product typically involves the use of traditional industrial hygiene sorbent tubes to trap organic vapors present in the air as a pump draws them across the sorbent media. Subsequent laboratory analysis includes liquid extraction methods to prepare samples for analysis. Preliminary review of the Navy Environmental Health Center's (NEHC) database for industrial hygiene sampling during use of Formula 150 primer revealed an abundance of sampling for *n*-butanol and a lack of sampling for other potential components.

Both VX and sarin are organophosphate nerve agents of concern because of their high acute mammalian toxicity and their potential use by military and terrorist groups. These two compounds were selected for examination not only because of their high toxicity but also because they provided the opportunity to examine the capability of the SPME-GC/MS system to sample and analyze compounds of widely differing volatilities. With VX being the least volatile of the nerve agents and sarin one of the most volatile agents, each presented unique challenges regarding sampling and analysis.

The use of nerve agents in warfare has been limited. However, Iraq's use of nerve and mustard agents in the Iran-Iraq war has been documented and the use of chemical weapons against their Kurdish population has been alleged [5]. In 1995, the religious cult, Aum

Shinyriko, used Sarin in an act of terrorism, killing 12 and injuring numerous others in a Tokyo subway [5].

The physiochemical properties and toxicological data for these nerve agents are provided in Table 1. While being a relatively non-volatile liquid, VX, with a LD_{50} of 10 mg/70 kg can easily be regarded as the most toxic of the Chemical Warfare Agents (CWA). When dispersed as a CWA, VX will likely be disseminated in the form of droplets from a spray device or various types of munitions. After release, the droplets will settle, contaminating the environment. Once on the ground, VX does not readily re-enter the atmosphere because of its low vapor pressure and, based upon its Henry's Law Constant, volatilization from moist soil is unlikely. Furthermore, with a soil sorption constant (K_{oc}) of 640, VX mobility in the environment is expected to be minimal. Because of these physiochemical properties VX is considered a persistent CWA.

The same properties that make VX a persistent CWA also make it a difficult agent to detect in the environment using traditional air sampling and analytical methods. To further complicate identification, VX is lost to the environment through degradation and possibly through sorption to organic material in some soils. The rate of loss is dependent upon a number of factors including the type of soil present, and presence or absence of moisture and vegetation. The VX lost to degradation, primarily through hydrolysis, can be identified in the environment as a

number of organophosphorus compounds. The primary degradation product however, is the compound bis (diisopropylaminoethyl)disulfide $(DES)_2$ [8]. The identification of this, or other stable degradation products, should be considered as a potential marker for the presence of VX.

Property	VX	Sarin (GB)
Chemical formula	$C_{11}H_{26}NO_2PS$	$C_4H_{10}FO_2P$
Molecular weight	267.4	140.1
Estimated human toxicity[6] LD_{50} mg/70kg person (percutaneous)	10	1700
Vapor pressure[7] mm Hg@ 25 °C	0.0007	2.9
Volatility (mg/m^3)[7]	10.5	22,000
Henry's Law constant[7] (H, atm x m^3/mol)	3.5×10^{-9}	5.4×10^{-7}
Log K_{ow}[7]	2.09	0.299
Log K_{oc}[7]	2.5	1.77

Table 1-1. Physical and chemical properties of VX and sarin.

While it is generally regarded as less toxic than VX, sarin is one of the most volatile and toxic of the G nerve agents. Because of its physiochemical properties, it is considered a non-persistent agent. Sarin tends to exist in the vapor phase, if released to the air, due its relatively high vapor pressure. If released to soil, sarin should be expected to demonstrate mobility based upon its K_{oc}. On dry soil, sarin will volatilize; however, its Henry's Law constant indicates volatilization from moist soil or water should not be expected.

Conclusion

Presidential Review Directive (PRD) 5 established objectives for the development of simple and effective methods to assess troop exposures to environmental pollutants [9] whether they are chemical warfare agents or toxic industrial chemicals. This is to be accomplished through development of smaller, lighter, more sensitive, rugged personal and area samplers and detectors capable of measuring multiple exposures/chemicals at toxicologically relevant points [9]. The work done in the following studies accomplishes many of these objectives and will help the U.S. military meet the requirements of PRD 5. Furthermore, it will have broad application in civilian industrial and emergency response communities and in homeland security.

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CHAPTER 2

SOLID PHASE MICROEXTRACTION FOR RAPID FIELD SAMPLING AND ANALYSIS BY GAS CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS)

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ABSTRACT

Modern gas chromatography/mass spectrometry (GC/MS) methods and equipment, and the sensitivity and structural information these methods provide make GC/MS an excellent choice for field detection and identification of a range of organic chemicals. Numerous sampling techniques allow detection of GC/MS analytes in environmental matrices, although multiple sample handling steps and use of extraction solvents increase the complexity and time needed to complete analyses. Solid phase microextraction (SPME) has been shown to be suitable for sampling environmental contaminants from air, water, and soil for GC/MS analysis. We provide applied examples of environmental samples collected and analyzed in the field using SPME-GC/MS for qualitative identification of workplace air contaminants from a poorly characterized paint, and in identifying gas phase contaminants present during forensic

and clean-up operations following a large fire involving aircraft fuel. In both instances, passive SPME sampling concentrated analytes from the air following short sampling periods and was followed immediately by GC/MS analysis in the field, without further sample preparation. The SPME sampling method is attractive for field use owing to its portability, simplicity of use, broad applications, sensitivity, and favorable attributes as a sample introduction method for GC/MS analyses.

KEY WORDS: Solid phase microextraction, gas chromatography, mass spectrometry, field detection

INTRODUCTION

In both the civilian and military communities, there is growing demand for rapid field analysis of volatile and semi-volatile organic compounds [1,2,3,4]. Analytical instrumentation for detection and identification of these compounds has become smaller, more reliable, and increasingly sensitive. Gas chromatography (GC) tools have undergone important improvements such as development of open tubular columns with bonded stationary phase material, providing improved chromatography and decreased fragility compared to packed

column GC. Mass spectrometry hardware for electron impact (EI) mass spectrometry (MS) has grown smaller and increasingly sensitive.

While these technological improvements in hardware have made field GC/MS analysis possible, the sampling and sample preparation methods for these compounds have essentially remained unchanged and continue to rely upon the proven and reliable techniques. These methods do not easily support rapid sampling and analysis carried out completely, or mostly, in the field. Traditional sampling methods include the trapping of analytes on a sorbent media during active air sampling, taking bulk air samples with Tedlar bags or summa canisters, and bulk samples of soil or water. While collection of air samples on sorbent media affords the ability to detect the target compounds at low levels, a logistical burden is imposed by the use of sampling pumps or other equipment. In addition, these samples must be prepared for introduction to an analytical instrument for analysis. The major methods for preparing an environmental sample for analysis are liquid-liquid, gas-liquid, solvent, gas-phase and supercritical fluid extractions [5]. These preparation methods are time consuming and require the use of additional analytical equipment and hazardous materials.

SPME is a technique that is well suited for field sampling [1, 3, 6]. It is a solvent free process that combines sampling, extraction, concentration and instrument introduction into a single step, eliminating the

complicated sample preparations methods described previously [7-9]. SPME passively extracts organic compounds and concentrates them onto a thin, fused silica fiber coated with a stationary phase material [7].

There are three different extraction modes for SPME - direct, headspace and membrane [7]. In the direct extraction mode, the fiber is placed in the water or air sample and the analytes are adsorbed on to or absorbed into the fiber coating directly from the sample matrix. In the headspace mode, a sample of soil or water is placed into a vial. The SPME fiber is placed in the air directly above the water or soil and analytes partition from the sample matrix through the air to the fiber coating. The air in the vial serves as a barrier between the SPME fiber and the sample matrix to protect the SPME fiber and eliminate fouling by high molecular weight compounds and other nonvolatile interferences in the sample media [7,10]. The third SPME extraction mode uses a membrane to protect the SPME fiber from heavily polluted samples that may damage the fiber.

Once an extraction is complete, SPME allows rapid transfer to an analytical instrument of choice [11] where the analyte is usually thermally desorbed, i.e. in the injection port of a GC system. Use of SPME can potentially eliminate the need for time-consuming sample preparation steps required by traditional sampling methods. If SPME can be used for a

given application, the need to carry hazardous solvents in the field is reduced or eliminated.

SPME methods have been developed to solve a wide variety of problems, including clinical, forensic, food and environmental applications [12,13]. It has been shown to have potential as a rapid air sampling method for volatile organic compounds (VOC's) [14,15] and as a method for time-weighted average air sampling [16,17]. SPME methods for analysis of organic contaminants in water and soil have received extensive attention and demonstrated much utility for VOC's, pesticides, polychlorinated biphenyls, organo-metallic compounds, and chemical warfare agents [7,9, 18-24].

While field sampling with SPME followed by laboratory analysis is well documented, relatively little has been published regarding the use of SPME with immediate analysis in the field. Gorecki and Pawliszyn [3] have demonstrated SPME is a viable sample introduction method for high-speed GC separations in the field. Koziel *et al.* [25] used SPME for field sampling with laboratory analysis to detect formaldehyde in indoor air, with on-fiber derivitization. Koziel *et al.* [26] and Jia *et al.* [27] used SPME to sample and analyze a number of organic analytes in the field. Field analyses of the preceding work mentioned in this paragraph were completed using non-orthogonal detectors. For field sampling using SPME and analysis completed in the field by GC/MS, Smith *et al.* [6] sampled

thermal degradation products from high temperature dispersion of CS riot control agent, successfully identifying by mass spectrum match, a number of compounds that would have been missed using a solvent delay for mass spectrometer startup, as needed for analysis of typical sampling tube solvent extracts.

We have used SPME coupled with GC/MS as a rapid field screening method to provide sensitive detection and identification of organic compounds from complex mixtures. We show the usefulness of SPME-GC/MS for qualitatively analyzing poorly characterized contaminants from air sampled during application of a marine coating onboard ship, and during emergency response following a large structural fire involving aviation fuel. The shipboard sampling provided qualitative data concerning substituted benzene compounds present at high concentrations in the paint used. The presence of these compounds was not identified in relevant ingredient lists or in material safety data sheets. With a sample of the aviation fuel involved in the fire, the field sampling and analysis at the fire scene confirmed the presence of this material in the air.

MATERIALS AND METHODS

Materials

All SPME fibers and holders used in this study were commercially available from Supelco (Bellefonte, PA). Prior to use, each fiber was conditioned following the manufacturer's recommendations. To ensure there was no carryover of analytes from previous extractions, blank runs were completed at least once daily before use of any fibers for sampling.

The marine coating sampled during routine ship maintenance is a paint primer manufactured by Niles Chemical Paint Company (Niles, MI). The *o*-(reagent grade), *m*-(certified grade) and *p*-xylene (99.8%) standards used to confirm analyte identification were purchased from Fisher Scientific (Pittsburgh, PA). The following standards were purchased from Aldrich (Milwaukee, WI), *n*-butanol (99.5%); propylbenzene (98%); 2-ethyltoluene (99%), 3-ethyltoluene (99%), 4-ethyltoluene (90%); 1,2,3-trimethylbenzene (90%); 1,2,4- trimethylbenzene (98%); 1,3,5-trimethylbenzene (97%); nonane (99%); decane (99%); undecane (99%); dodecane (99%); tridecane (99%); tetradecane (99%); pentadecane (99%); naphthalene (99%); biphenyl (99.5%); phenanthrene (98%); and anthracene (99%). In the case of samples collected at the fire scene, a clean sample of the aircraft fuel (jet A) involved was obtained from law enforcement investigators for analysis and comparison to the field sample results.

Sampling

The paint samples were collected as general area samples by placing the SPME holders near the center of the roller painting operation at approximately 5 feet above the floor. A single individual was applying paint to a passageway of approximately 300ft^2 during sampling. Anticipating the presence of volatile compounds that would be rapidly absorbed, it was estimated that a 10 min sampling time would provide adequate sensitivity for qualitative analysis.

The paint-related samples were collected using 100 μm thickness polydimethylsiloxane (PDMS) fibers for several reasons. PDMS is a durable, nonpolar phase coating capable of enduring injector temperature of up to 300°C. Its nonpolar characteristic favors extraction of nonpolar analytes, which, based upon some previous experience with this type of product, were anticipated. The PDMS coating is also capable of extracting some increasingly polar analytes. The 100 μm thickness was selected over thinner coatings as it allows for a greater mass of analytes to absorb into the fiber coating, provided increased sampling times are used.

The samples obtained at the fire scene were also area samples; however, these fibers were exposed to the air for 30 min. The potential air contaminants at the fire scene could not be as readily anticipated as they were with the painting operation; therefore, a longer sampling time

was used to help ensure adequate sensitivity was achieved for the qualitative analysis. The primary fire had been extinguished for one day when sampling commenced. Occasional re-flash fires occurred during the recovery and investigation period. As the air contaminants present in this instance were essentially completely unknown, 4 types of fibers were used to take advantage of fiber type selectivity differences. PDMS fibers were used for nonpolar analytes; Polyacrylate (PA) and Carbowax/divinylbenzene (CW/DVB) fibers for polar analytes; and Carboxen/PDMS fibers for mixed polarity analytes in the C_2-C_{12} range [28]. Following initial sampling and analysis with this array of fibers, CW/DVB and PDMS fibers exhibited the greatest sensitivity for the analytes observed. Therefore, these two fibers were used to collect a series of samples, beginning in habitable areas of the building and culminating at the heart of the fire scene. In both areas of this qualitative effort, worst case air samples were desired. In the habitable areas, this was obtained by placing the SPME fibers in locations closest to the fire damage and where smoke damage appeared the greatest. In the heart of the fire scene, the fibers were located at places where active recovery and investigation operations were taking place. All fibers were located 3 to 5 five above the floor when sampling. The exact locations were chosen to allow placement as close to workers as possible without impeding their progress, yet secure enough to ensure the delicate SPME fiber was not

broken by the physical labor involved in the recovery and investigation effort.

For qualitative identification and retention time match of bulk paint, and fire scene-related bulk fuel samples, as well as samples of single compound standards, headspace sampling was completed in 15 ml glass vials with PTFE/silicone septa (Supelco). For these standard comparison samples, fiber exposure time was determined empirically. Typically, analyte peaks for these standard samples were quite large with sample times as short as 5-10 s. Between collection of field samples and analysis (10-30 min in all cases), the tip of the SPME fiber was retracted into the protective sheath. The sheath was then inserted into a Thermogreen LB-2 septum (Supelco) to minimize further extraction onto or loss of analytes off of the SPME fiber.

Fiber-kinetic studies on several important analytes from the paint sample were performed in the laboratory using the same analytical equipment used in the field. Uptake curves were completed by exposing the SPME fiber to equal concentrations of the standards in the headspace of a vial for periods of time ranging from 5 s to 10 min. The fiber uptake samples were run in triplicate.

Fiber selectivity data were collected in a side-by-side study of naphthalene and pentadecane. PDMS and CW/DVB fibers were exposed to 6.2 and 7.7 μg of naphthalene and pentadecane respectively

in HPLC grade methylene chloride (Fisher Scientific) for 30 min in 15 mL vials. To determine if there was a statistical difference in the response of naphthalene and pentadecane to the PDMS and CW/DVB fibers, a comparison of the mean responses for each analyte on a given fiber was performed using a 2-tailed T test (2 tests total, 1 test per fiber).

The mass spectrometer relative response factor for *n*-butanol, 3-ethyltoluene, and 1,2,4-trimethylbenzene was evaluated by directly injecting equal masses of the analytes into the GC/MS injection port. For 3-ethyltoluene and 1,2,4-trimethylbenzene (density 0.81 g/mL and 0.87 g/mL respectively), 1μl of each standard was diluted in 10 mL of toluene (HPLC grade, Fisher Scientific). As *n*-butanol elutes at essentially the same time as toluene, 1 μl of butanol was diluted in 10 mL of mesitylene (97%, Aldrich). This was performed to rule out MS response difference for the analytes as a possible explanation for the apparent *n*-butanol sampling-selectivity difference when compared with the substituted benzene compounds that were noted in the uptake curves. Samples for the response factor and fiber selectivity were run in duplicate.

GC/MS Methods

For paint-related analyses performed in the field, the SPME fiber samples were desorbed thermally in the injection port of a portable Viking Spectra Trak 572 GC/MS system (Bruker Daltonics, Billerica MA) onboard

the ship where the painting occurred, within 10 min of completion of sampling.

Fire-related samples were analyzed in the field within 100 m of the worksite using the same SPME sample introduction technique. These samples were analyzed using a field portable Viking Spectra Trak 573 GC/MS system within 30 minutes of completion of sampling.

For both instruments, the injection port as used for SPME samples was equipped with a deactivated injection port liner designed for thermal desorption of analytes from a SPME fiber (0.75 mm I.D., Supelco). For analyses of paint-related samples, a 30 m x 0.250 mm I.D. DB1-MS column (0.25 μm film thickness, J&W Scientific, Folsom CA) was used with He carrier gas and an initial linear velocity of 47 cm/s. Temperatures were: 175 °C (injection port and transfer line), 90 °C (MS transfer line), and 195 °C (MS ion source). GC oven temperature began at 35 °C, was held there for 5 min, then increased at 1 °C/min to 46 °C, and then at 3 °C/min to 75 °C. Split injection (50 mL/min split flow) was used to improve the ability to resolve several important peaks in these samples. EI (70eV) ionization was used and mass spectra were collected over 30-350 mass-to-charge ratio (*m/z*) range.

For analyses of air samples at the fire scene with the 573 instrument, a 20 m x 0.180 mm I.D. DB-5 column was used (x 0.18 μm film thickness, J&W Scientific). Carrier gas was He with an initial velocity of 35 cm/s. The

injection port and injector transfer line were maintained at 275 °C throughout the analysis. The GC oven temperature began at and was held at 35 °C for 1 min, and then increased at 20 °C/min to 275 °C. These analyses were performed in splitless injection mode, with split flow (30 mL/min) started at 2.00 min. The MS transfer line was maintained at 290 °C. EI (70 eV) ionization was used and mass spectra were collected over the range 35-350 *m/z* operating with quadrupole and ion source temperatures of 106 and 230 °C respectively.

Because no solvent is used in SPME introduction of samples into the GC/MS inlet, the typical solvent delay for startup of MS data collection was not required for analysis of field SPME samples from either location. Peak area for all quantitative comparisons were determined using MS Chemstation chromatogram integration software (Agilent Technologies, Palo Alto, CA, USA).

The field portable GC-MS systems used are built around Hewlett Packard (now known as Agilent) quadrupole mass spectrometers – a Hewlett Packard 5972 monolithic quadrapole and associated source, and electron multiplier, in the case of the 572 instrument, and an Aglient Technologies 5973 monolithic quadrupole and associated source, and electron multiplier, in the case of the 573 instrument. The mass spectrometer in the 573 instrument thus carries the product improvements that differentiate the 5973 from the 5972 mass spectrometer. The other

capabilities of the complete Viking 572 and Bruker-Viking 573 instruments are similar. A description of the Viking 572 and its capabilities has been provided by Eckenrode [29].

RESULTS AND DISCUSSION

Upon completion of initial analyses, the paint-related SPME samples showed the presence of a number of substituted benzene compounds. These compounds were not identified in material safety data sheets (MSDS) or in ingredient lists and therefore, it is unlikely industrial hygienists monitor for exposure to these compounds. As completed to that point, the analyses essentially provided a field screening method using only the National Institute of Standards and Technology (NIST) mass spectral library software [30] for tentative identification. Without access to standards or elution order data, the substituted benzene compounds observed are (within a given group of isomers) poorly distinguished based solely on mass spectra. Further study in the laboratory with purchased standards confirmed the peak identities observed in Figure 2-1.

Uptake curves, Figure 2-2, for four of the principle analytes observed - 1,2,4-trimethybenzene (1,2,4 TMB), 3-ethyltoluene, 4-ethyltoluene, and n-butanol - show n-butanol reached equilibrium almost immediately while

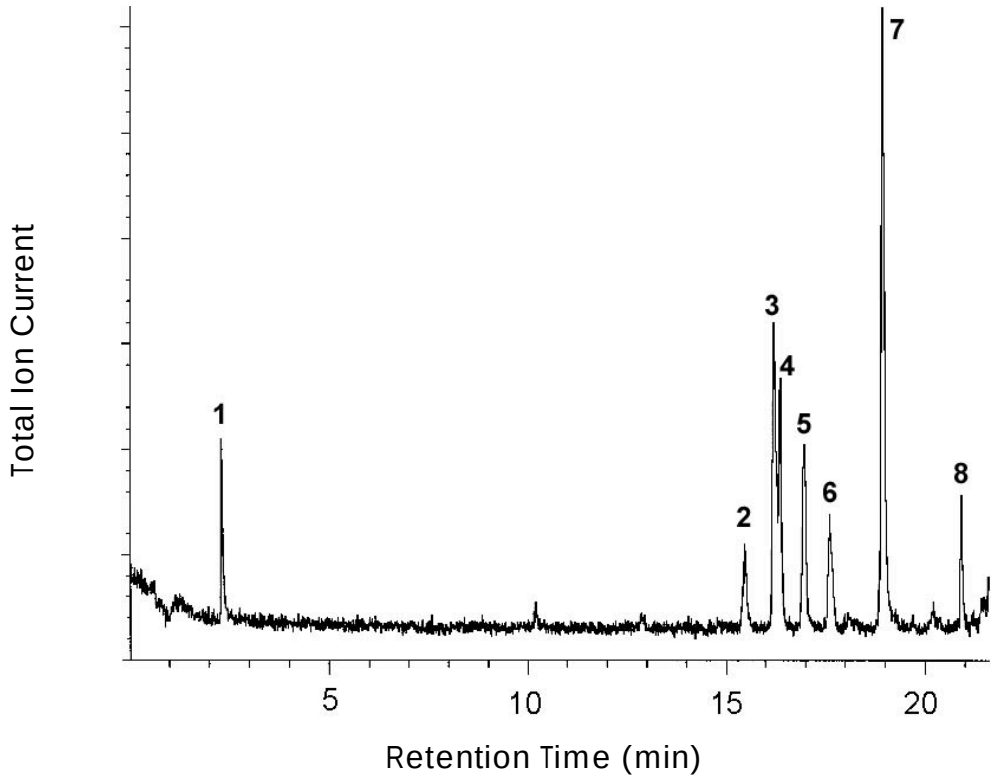

Figure 2-1. SPME-GC/MS chromatogram, sample collected during shipboard painting with field analysis. Compound key: 1 *n*-butanol, 2 propylbenzene, 3 3-ethyltoluene, 4 4-ethyltoluene, 5 1,2,3-trimethylbenzene, 6 2-ethyltoluene, 7 1,2,4-trimethylbenzene, 8 1,3,5-trimethylbenzene.

1,2,4, TMB area counts were still slowly increasing at 10 min. The nonpolar PDMS fiber had a greater capacity for the nonpolar 1,2,4 TMB and required longer to reach equilibrium compared to the more polar n-butanol. Based upon the data presented in Figure 2-2, the 10 min duration of the field screening sample adequately balanced sensitivity with efficient use of time to characterize qualitatively the exposure to the nonpolar substituted benzene paint components.

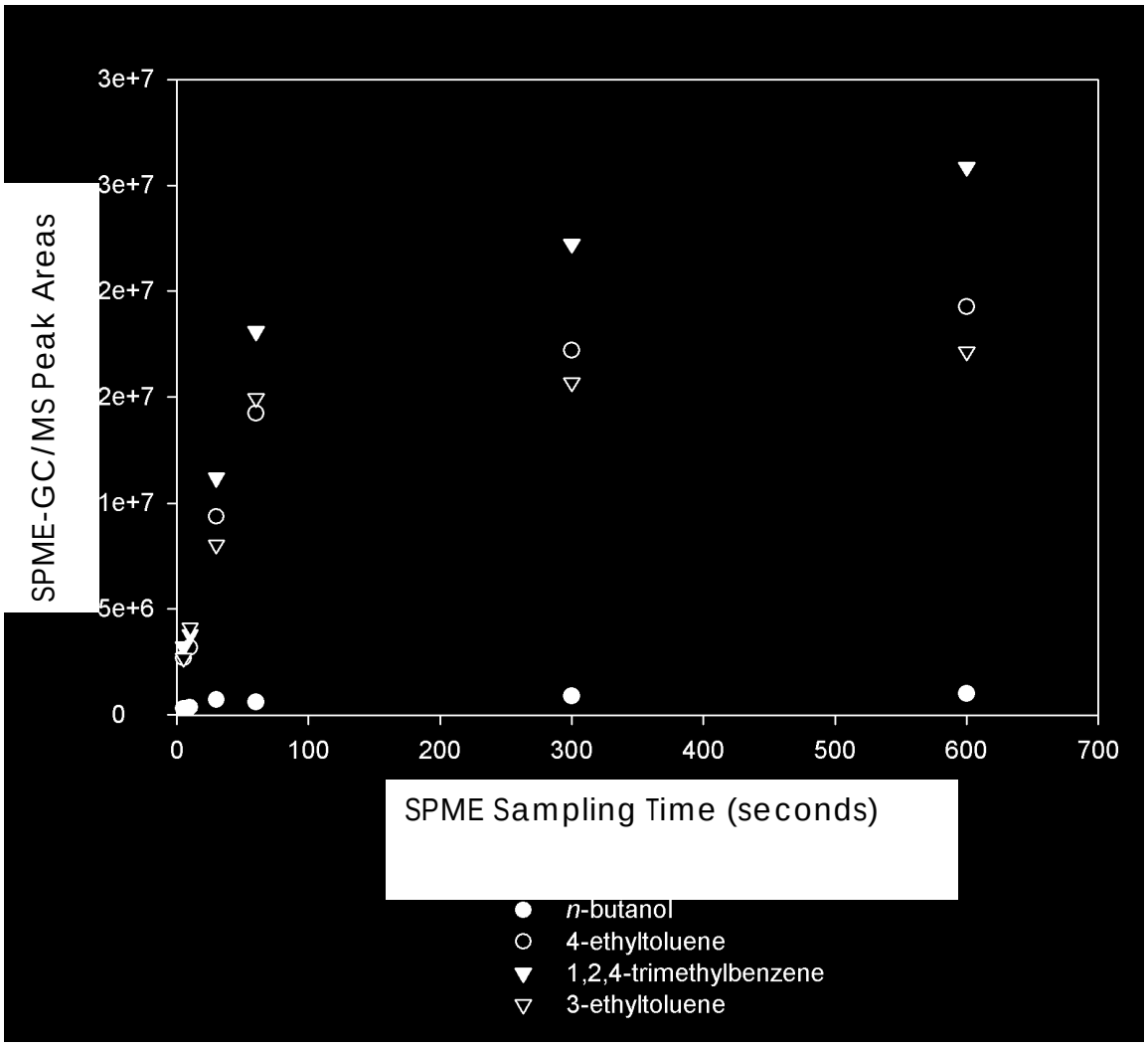

Figure 2-2. Uptake curve for 1,2,4-trimethylbenzene, *n*-butanol, 3-ethyltoluene, and 4-ethyltoluene, 100 μm PDMS fiber

The direct injection of *n*-butanol, 3-ethyltoluene, and 1,2,4-TMB showed the MS had a greater response per mass analyte injected for *n*-butanol than the other two analytes. The MS response for 3-ethyltoluene and 1,2,4-TMB were similar. Therefore, the very large difference in the n-butanol response seen in Figure 2-2 when compared to the response of

the other analytes is a result of differing SPME uptake characteristics of the PDMS fiber used and not due to differing response by the MS.

Samples from within the site of the fire demonstrated the presence of a rich mixture of aliphatic and aromatic hydrocarbons, with the chromatogram shown in Figure 2-3(a) exemplifying these samples. The identification of aliphatic hydrocarbons such as tetradecane and pentadecane, and the various volatile polycyclic aromatic hydrocarbons suggested the presence of aviation fuel components as air contaminants. A 30 min headspace sample of a 1:320 dilution of the bulk aviation fuel involved in the fire confirmed the source of the compounds present in the fire scene air (Figure 2- 3(b)).

Side by side fire scene samples indicated CW/DVB fibers had a greater affinity for sampling the aromatic compounds studied than for long chain hydrocarbons. Table 2-1 summarizes fiber selectivity differences for naphthalene and pentadecane, as studied in the laboratory. The PDMS fiber gave a statistically indistinguishable response to both compounds, while the CW/DVB fiber had greater affinity for the aromatic analyte ($p<0.05$, 2-tailed T-test). The selectivity of a fiber for a given analyte must be considered when evaluating unknown samples and when quantifying analytes. The relative abundance of a given analyte and hence, sensitivity, can change dramatically between various fiber types. Disregard for the fiber phase can result in inappropriate

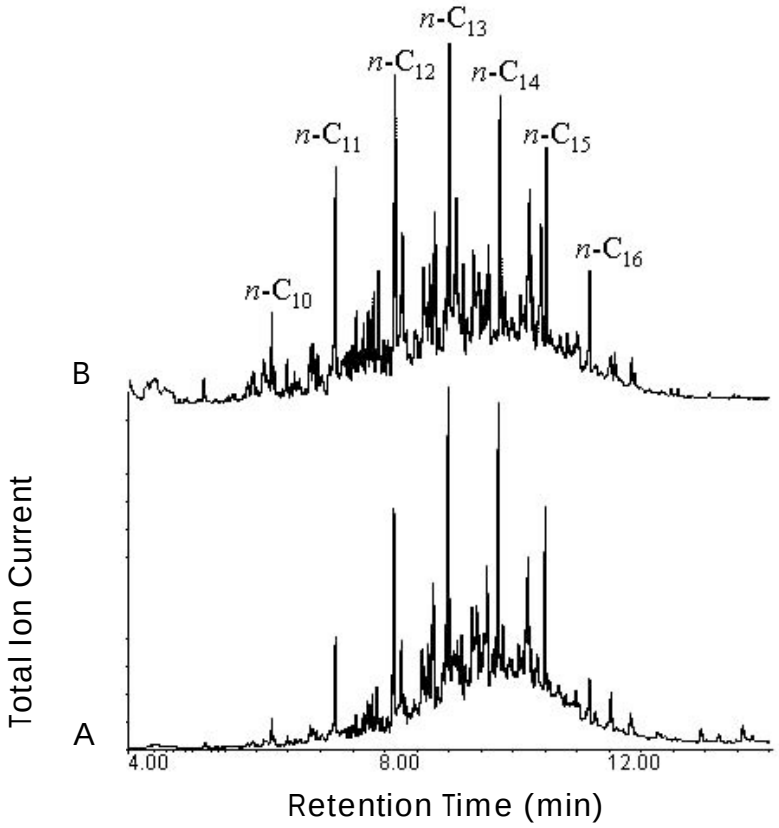

Figure 2-3. (a) Fire scene sample, (b) standard sample from diluted aviation fuel (320:1 $MeCl_2$ diluent)

TABLE 2-1. Fiber Selectivity

Fiber	Mean Peak Area	[1]S	[2]RSD(%)
CW/DVB			
Napthalene	8.2×10^8	3.9×10^7	4.8
Pentadecane	4.5×10^8	1.2×10^7	2.8
PDMS			
Napthalene	7.1×10^9	1.2×10^8	1.8
Pentadecane	7.0×10^9	1.3×10^8	1.8

1. S = Standard Deviation
2. RSD = Relative Standard Deviation

dismissal of an apparently insignificant peak during qualitative screening which, in reality, may appear to be insignificant only because of a low affinity between the analyte and the fiber phase used for the screening extraction. For obvious reasons, maximum sensitivity is desirable when aiming to quantitate analytes. Therefore, use of multiple fibers of varying polarities would be prudent for screening unknown samples.

In the case of the fire scene samples, the rapid data (analysis complete <1 h past starting sample collection) provided by the SPME-GC/MS field screening was used to guide quantitative sampling performed in a fixed laboratory that provided results no earlier than 1 day following sample submission.

When using SPME for quantitative analysis, prior knowledge of the analytes of interest present in the sample is helpful in order to select the proper fiber phase, sample volume, extraction time, extraction conditions, and desorption conditions. Quantification is challenging when performing field screening of unknown analytes. For analytes that may be sampled using an adsorptive SPME coating, active sampling with diffusion-based calibration provides major improvements in areas such as sensitivity, precision, and the ability to quantify known analytes without typical SPME calibration curves, but only if detector response factors are known for the analytes in question [15]. A drawback to the diffusion-based calibration

method is that it is not passive, adding additional equipment and complexity to field sample collection.

CONCLUSIONS

SPME was used as a sampling and sample preparation method for on-site field GC/MS. Its simplicity of operation, sensitivity, selectivity, portability, and the solvent-free nature of the method make it a powerful tool for sampling and sample introduction for field GC/MS screening of airborne organic chemicals. This work included both chemicals that are poorly defined in their MSDS as well as completely unknown samples in the field. Additional work should continue to explore the usefulness of sample concentration with SPME coupled with field portable GC/MS to provide near real-time screening/identification of poorly defined and unknown analytes. The use of SPME-GC/MS completed in the field can serve to provide monitoring guidance for traditional occupational and environmental exposures as well as in emergency response. Resources for quantitative exposure monitoring can be more efficiently employed if accurate qualitative information is rapidly available.

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CHAPTER 3

SOLID PHASE MICROEXTRACTION SAMPLING AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY FOR FIELD DETECTION OF THE CHEMICAL WARFARE AGENT O-ETHYL S-(2-DIISOPROPYLAMINOETHYL) METHYLPHOSPHONOTHIOLATE (VX)

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ABSTRACT

A rapid detection method for the chemical warfare nerve agent VX was developed using solid phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS). Five commercially available SPME fiber coatings were evaluated to determine the optimal fiber coating and conditions for extraction. The use of silanized vials was found to be necessary to limit interaction of the basic tertiary amine component of VX with the acidic silanols present in standard glass vials. The polydimethylsiloxane (PDMS) fiber was ultimately selected for

completion of this work with extractions being performed at 50 °C. Clothing material was spiked in the laboratory with 1 μL of neat VX, placed in a sealed vial and taken into the field for SPME sampling, using optimized conditions studied. GC/MS analysis was also completed in the field using van-mounted instrumentation. With sampling and analysis completed in less than 20.0 minutes, detection of VX contamination was relatively rapid, especially considering the quality of the resulting data. The use of SPME also provides increased safety for the field analysis of VX since it does not require the handling of solvents for sample preparation and samples are not handled directly by the analyst.

KEY WORDS: VX; solid phase microextraction; field analysis; gas chromatography; mass spectrometry

INTRODUCTION

Rapid and reliable detection methods are needed for high-concern analytes such as chemical warfare agents (CWA). This is driven from the need to detect the potential presence of CWAs in both civil and military settings. Current methods for detecting CWAs in the field include commercially available detector tubes and the military's colorimetric kits, ion mobility spectrometry monitors, and infrared systems for optical

remote sensing [1]. These are screening methods that only indicate the potential presence of CWAs and are subject to false positive readings. Following a positive test using a screening method, confirmatory analysis is typically performed in a laboratory using GC or liquid chromatography separation followed by a spectrometric technique such as infrared, nuclear magnetic resonance or mass spectrometry [2,3]. There is increasing demand for high quality analytical data provided in the field [4-7], which is of obvious benefit for deployed military units and civil emergency response organizations.

Technological advances have made field GC/MS analysis more practical; however, traditional sample preparation procedures can take more time than an actual analysis and require the transportation, storage, use and disposition of hazardous solvents in the field. SPME simplifies and speeds up the sampling/sample preparation steps and reduces or eliminates the requirement for solvents in the field. Hook *et al.* [8] and Smith *et al.* [9] have shown SPME to be a technique that can be used for GC/MS analysis in the field. A thorough review of SPME background and methodologies has been provided in references [10] and [11].

A number of studies have been completed regarding the use of SPME to sample CWAs in air and water. Schneider *et al.* [12] developed a method to screen air and water with SPME followed by laboratory GC/MS analysis for the volatile nerve agent sarin. Smith *et al.* [13] developed a

SPME field sampling method for airborne hydrogen cyanide with laboratory GC analysis and nitrogen-phosphorous detection (NPD). Lakso and Ng [14] compared the use of SPME-GC/NPD with liquid-liquid extraction for detection of the nerve agents sarin, soman, tabun and O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate (VX) in water. Sng and Ng [15] developed a procedure for SPME-GC/MS detection of CWA degradation products in water. Alcaraz *et al.* [16] used SPME to obtain samples for a variety of chemicals controlled by the Chemical Weapons Convention from sea water, canal water, and soil leachate for field analysis by GC and flame photometric detection and GC/MS. Headspace SPME has been used for sampling the CWA sulfur mustard on soil [17].

The need for reliable field identification of VX exists because of its potential use as a military or terrorist weapon, and due to its high human toxicity. Based upon its toxicology, a strong argument can be made for VX being the most toxic chemical warfare agent with an estimated percutaneous lowest lethal dose in man of 70 μg/kg [18]. Furthermore, in comparisons of acute toxicity with the nerve agents tabun, sarin, and soman, VX is one of the most toxic nerve agents via a wide variety of routes of administration and species [19]. Its low vapor pressure of 0.0007 mm Hg at 25 °C and its stability make VX relatively persistent.

This work evaluates the potential of headspace SPME-GC/MS for field detection of VX. The low volatility of this analyte introduces additional challenges in successfully completing headspace SPME sampling, compared with other more volatile CWAs. A useful SPME-GC/MS field method for this analyte will greatly lessen the potential analyst exposure to VX, and will allow rapid sampling and give high confidence in analytical results when VX is detected. Rapidly available, high quality data following the accidental or intentional release of a high concern chemical, in either a military or civilian setting, provide the information needed for making an appropriate response regarding issues such as clean-up and medical treatment requirements.

Initially, laboratory evaluations were made to answer questions concerning SPME fiber selectivity and uptake kinetics issues. This was followed by detection of VX contamination on clothing material in a field setting using SPME-GC/MS.

MATERIALS AND METHODS

Materials

For the laboratory work, VX (95% purity) diluted in chloroform to 0.9 mg/mL was obtained from the U.S. Army Edgewood Chemical Biological Center (Aberdeen Proving Grounds, MD). For the field work, VX (97%

purity) was obtained from Defence Research and Development Canada - Suffield (Medicine Hat, Alberta, Canada). A standard for bis(diisopropylaminoethyl)sulfide was produced by reacting 2-(diisopropylamino)ethyl chloride hydrochloride (Aldrich, Milwaukee, WI) with two equivalents of potassium thioacetate in acetonitrile. The thioacetate was purified and reacted with ammonia in methanol to generate 2-(diisopropylamino)ethanethiol, which was coupled with 2-(diisopropylamino)ethyl chloride hydrochloride in acetonitrile with potassium carbonate. A standard for bis(diisopropylaminoethyl)disulfide was produced by exposing a sample of 2-(diisopropylamino)ethanethiol to atmospheric oxygen for 12 hrs, yielding the corresponding disulfide. The disulfide was distinguishable from the thiol and the sulfide by thin layer chromatography, ^{1}H NMR and GC/MS with 70ev electron impact (EI) detection.

The following five SPME fiber coatings (Supelco, Bellefonte, PA) were evaluated: 100 μm polydimethylsiloxane (PDMS), 85 μm polyacrylate (PA), 65 μm carbowax/divinylbenzene (CW/DVB), 65 μm carboxen/polydimethylsiloxane (CAR/PDMS), and 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB). Each fiber was conditioned following the manufacturer's recommendations prior to use. Blank runs were completed a minimum of once daily before use of any fibers for sampling.

SPME Sampling

Selection of optimal fiber. Selection of the optimal SPME fiber from among those tested was accomplished by obtaining triplicate samples from 15 mL glass vials with PTFE-lined silicone septa (Supelco) or 15 mL silanized vials (Alltech, Deerfield, IL) having open screw top closures fitted with PTFE/silicone septa. Each vial was spiked with 5.0 μL of a standard solution (0.9 mg/mL VX in chloroform) using a 10.0 μL syringe (Hamilton, Reno NV). To enhance the reproducibility of the spikes, a solvent chase method was used in which 1 μL of chloroform was drawn into the syringe, followed by 0.5 μL of air, and then the measured aliquot of VX solution. A digitally controlled hot-block heater (Barnstead/Thermolyne, Dubuque, IA) was used to maintain the vial temperature at 50 °C during sampling. Prior to sampling, each vial was allowed to equilibrate in the hot-block for 10.0 min after which the septum was pierced with the SPME fiber assembly and the fiber extended into the vial for a 30.0 min extraction period. While it is unlikely the SPME extractions would have significantly altered the concentration of VX in the vial, vials were sampled only one time to eliminate the potential for loss from the system due to multiple entries through the septum.

At the end of the extraction period, the SPME fiber was retracted into its protective sheath, removed from the vial and immediately introduced into the heated GC injection port. The fiber was then lowered

into the midrange region of the heated injection port liner (0.75 mm I.D. deactivated glass, Supelco) and GC/MS analysis commenced. The fiber providing the greatest GC/MS peak areas was selected for further sampling and analysis optimization.

Selection of Optimal Temperature and Sampling Time. A separate set of spiked vials was analyzed to determine the effect of temperature on the extraction. Using the fiber selected as the optimal fiber (PDMS), these extractions were performed under the same conditions previously used except the temperature was maintained at 25, 50, 75, or 100 °C. Finally, the fiber was exposed at the optimal temperature over an increasing extraction time period to examine the fiber uptake kinetics for VX.

Clothing Material Headspace SPME. A standard, cotton fabric, military issue undershirt was cut into 5 cm^2 sections that were placed in 15 mL silanized vials in a laboratory setting (n = 3). The undershirt material in each respective vial was then spiked with 1.0 μl of neat VX and the vial was sealed using an open - top closure with PTFE-lined septum. Headspace sampling with a 100 μm PDMS SPME fiber was completed in the field the following day in a portable fume hood equipped with an activated charcoal filtering system. Sample times of 1.0 and 5.0 min were completed with the vials pre-heated to 50 °C for 5.0 min.

Statistical Analysis. Experimental data resulting from fiber selection, extraction temperature, and uptake curve extraction time analyses were examined for differences between VX GC/MS peak areas. The statistical test used was the analysis of variance (ANOVA), which was completed for each of the data sets. As appropriate, this was followed by Tukey's *post hoc* comparison method to evaluate the source of observed differences. To examine reproducibility, RSD values were calculated for laboratory samples, which were run in triplicate, except for temperature optimization samples, which were run in duplicate.

GC/MS Methods

The laboratory SPME samples were analyzed immediately following collection using a 6890 series gas chromatograph and 5973 quadrupole mass selective detector (Agilent Technologies, Wilmington, DE). The GC was fitted with a J & W Scientific (Folsom, CA) DB-5, 30m x 0.25 mm I.D. column having a film thickness of 0.25 µm. Helium at 1 mL/min was used as the carrier gas. The oven was programmed to increase from 40 to 250 °C at 20 °C per minute following a 2.0 min hold time at the initial temperature. Desorption of the SPME fiber samples was accomplished in the splitless injection mode for 2.0 min, followed by 50 mL/min injector purge. The injector temperature was maintained at 250 °C throughout an analysis, and the mass spectrometer transfer line was kept at 270 °C. EI

ionization (70 eV) was used for samples analyzed in the laboratory and in the field. Mass spectra were collected over the range of 35-350 m/z. Sample retention characteristics and mass spectra were stored using the Agilent Chemstation software package.

Analyses of SPME samples collected in the field were performed in the field using the same type of GC/MS used for the laboratory samples; however, the GC/MS system used was mounted in a van making it portable. Water electrolysis was used to generate high purity H_2 carrier gas. This instrument was fitted with an Agilent HP-5MS column (30m x 0.25mm I.D., 0.25 μm film thickness), and operating parameters were identical to those used in the laboratory analyses.

RESULTS AND DISCUSSION

The initial laboratory studies were hampered by poor reproducibility apparently due to the reaction of the VX with active sites in the non-silanized glass vials originally used. Silanols on surfaces are known to be relatively acidic functional groups and experimentally, dissociation constant (pKa) values from 1.5-10 have been measured [20]. Consequently, the basic tertiary amine on VX likely associates strongly with the acidic silanols on the glass, inhibiting vaporization of the molecule. In addition, the nature and number of silanol groups on glass may vary

among vials causing different amounts of VX to be retained. Silanization of the vials results in a surface lacking acidic silanols and therefore in less potential interaction with VX. Figure 3-1. demonstrates the variability of the results using standard glass vials for VX. Through the use of silanized vials, the RSD's were reduced to an acceptable range of 0.2 % to 14 %. The presence of peaks other than VX (peaks 1, 2, 4, and 5) in the chromatograms of Figure 3-1. is likely due to the purity of the VX used for those samples (95% stated purity). The identified compounds have been previously identified as VX impurities [21].

Fiber Selection. Table 3-1 provides the data obtained during the fiber selection experiments. Statistically, the PDMS, PA, CW/DVB, and PDMS/DVB fibers were found to provide the same response. CAR/PDMS provided significantly less sensitivity. The fiber of choice for completion of this work was the PDMS fiber because it provided good sensitivity; in addition, it is a relatively durable coating that has been well characterized in previous work. However, the PA, CW/DVB or PDMS/DVB fibers could have been used and would likely have provided similar results.

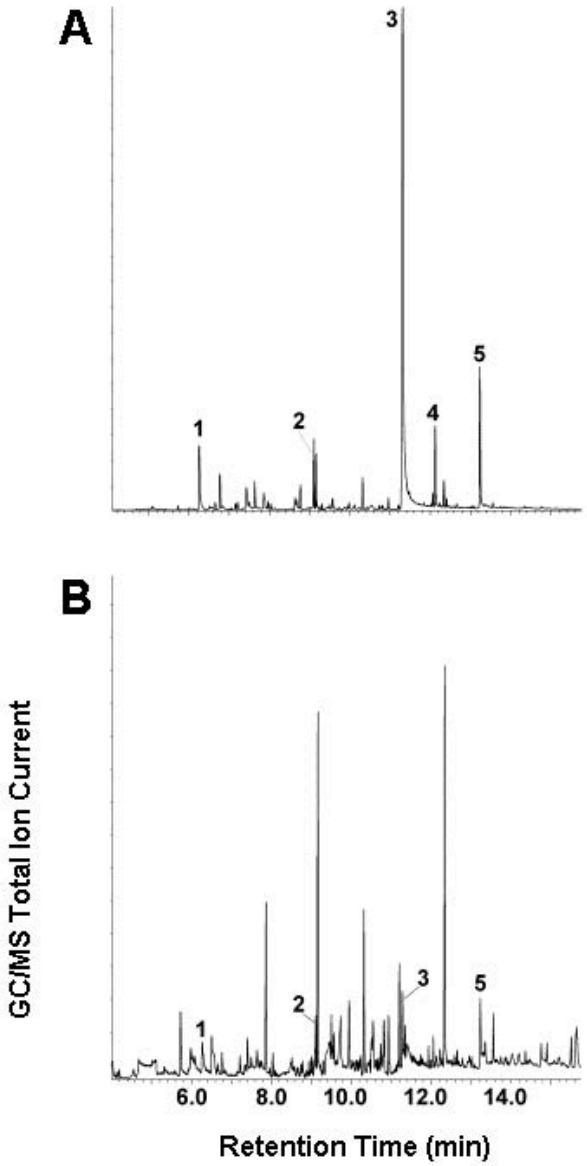

Figure 3-1. (a) and (b) Total ion chromatograms of 4.5 μg of VX sampled with a PDMS fiber for 30.0 min at 50 °C demonstrating the variability of results using unsilanized vials. Both samples were prepared identically and sampled from unsilanized vials. Compound key figure for Figures 1a, 1b, and 3: (1) Diethyl methanephosphonate[a)], (2) probable VX degradation product with 114 *m/z* ion, (3) VX[b)], (4) Bis(diisopropylaminoethyl)sulfide[b)], (5) Bis(diisopropylaminoethyl)disulfide[b)]

[a)]Identification based upon EI spectrum only

[b)]Identification based upon retention time and EI spectrum match with authentic standard

Table 3-1. Optimal Fiber Selection, GC/MS Peak Area Counts for VX, 30.0 min extraction, 50 °C

Sample #	PDMS	PA	CW/DVB	PDMS/DVB	CAR/PDMS
1	1.04×10^{10}	8.34×10^{9}	8.68×10^{9}	7.09×10^{9}	2.07×10^{9}
2	8.85×10^{9}	8.61×10^{9}	9.98×10^{9}	6.70×10^{9}	2.51×10^{9}
3	9.00×10^{9}	8.19×10^{9}	1.05×10^{10}	8.50×10^{9}	1.92×10^{9}
Mean	9.45×10^{9}	8.38×10^{9}	9.73×10^{9}	7.43×10^{9}	2.17×10^{9}
SD	8.98×10^{8}	2.10×10^{8}	9.54×10^{8}	9.50×10^{8}	3.04×10^{8}
RSD	9.51	2.52	9.80	12.80	14.06

Table 3–2. Optimal Temperature Selection, GC/MS Peak Area Counts for VX, 30.0 min extraction, PDMS fiber

Sample #	25 °C	50 °C	75 °C	100 °C
1	ND[a]	1.30×10^{9}	1.33×10^{9}	3.00×10^{8}
2	ND[a]	1.28×10^{9}	1.35×10^{9}	3.23×10^{8}
Mean	--	1.29×10^{9}	1.34×10^{9}	3.12×10^{8}
SD	--	1.34×10^{7}	1.03×10^{7}	1.66×10^{7}
RSD	--	1.04	1.03	5.32

[a]Non-detectable

Temperature and Sampling Time Selection. Table 3-2. provides the data resulting from temperature optimization experiments. VX was not detectable with sampling at 25 °C. The greatest sensitivity was achieved at 50 °C and 75 °C. The GC/MS response for samples taken at these temperatures was statistically indistinguishable. For the remainder of this work, samples were obtained at 50 °C.

Equilibrium was not obtained even after 60.0 min of sampling (Figure 3-2). Additional extractions with longer sampling times were not performed since 30.0 min extractions provided adequate sensitivity for this work.

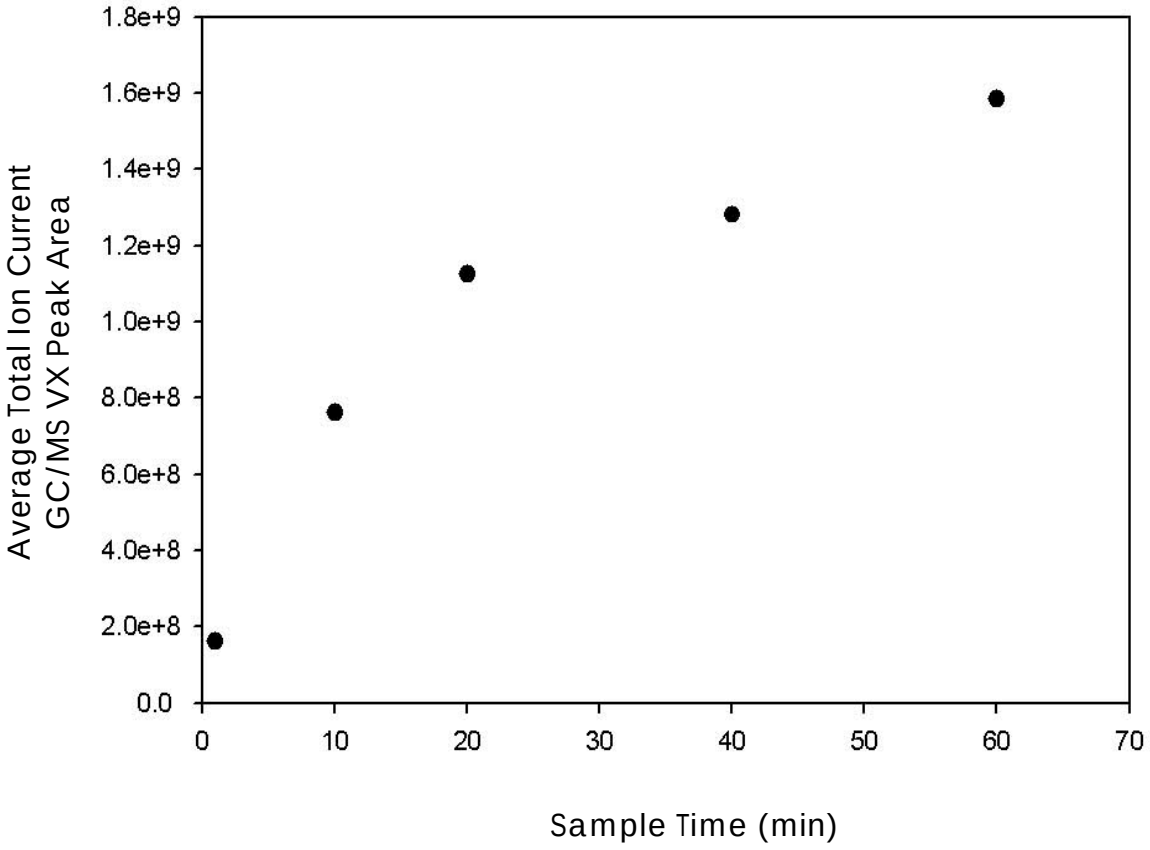

Figure 3-2. GC/MS total ion current peak area for VX plotted against SPME sampling time (PDMS fiber, 50 °C)

Clothing Material Headspace SPME. A 5.0 min headspace extraction of contaminated clothing at 50 °C allowed detection of VX. When the sampling time was reduced to 1.0 min (Figure 3-3), VX was still easily identified in the sample. In all clothing samples, the VX was readily detected with few signs of degradation observed. The use of traditional air sampling methods, such as sorbent tubes, would make detection of this contaminant difficult due to the low volatility of VX. Liquid extraction

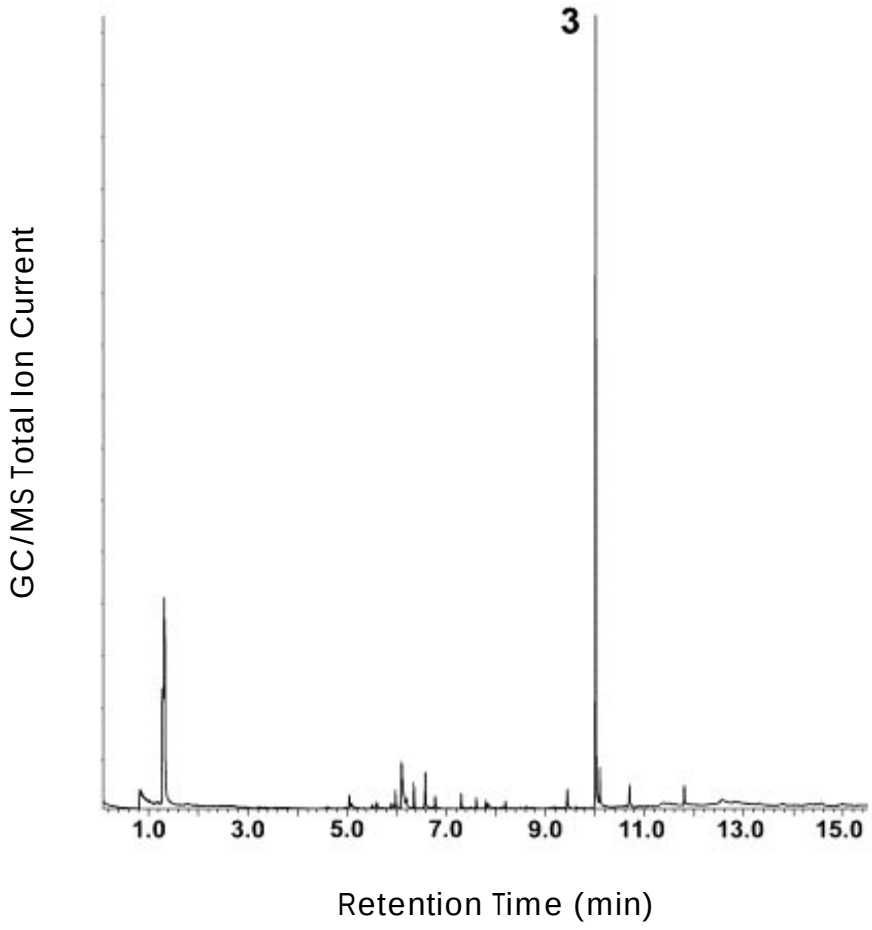

Figure 3-3. Field analysis, total ion chromatogram of 1.0 min, 50 °C, headspace PDMS SPME sample of VX contaminated clothing material.

could be used to detect VX from contaminated clothing; however, the use of solvents adversely impacts a sampling/analysis method to be carried into the field. Direct headspace sampling with a gas-tight syringe could possibly have been used to detect milligram quantities of VX contamination. However, unlike the SPME method, direct headspace sampling does not provide the benefit of concentrating the anayltes in a small volume for introduction into the GC injector. Concentration of the sample may be crucial for the detection of trace levels of contamination. The SPME method utilized allowed rapid headspace extraction of a low volatility compound by simply controlling the temperature of the sample media followed by immediate GC/MS analysis.

CONCLUSION

Due to its apparent reactivity, it was initially difficult to obtain reproducible data when using headspace SPME for sampling and analyzing VX. These results were improved through the use of silanized vials. The PDMS fiber coating and 50°C extraction temperature were selected for further use following optimization experiments.

By using sampling conditions optimized in the laboratory, headspace SPME with GC/MS in the field was shown to be a simple and viable method for confirming the presence of milligram quantity

contamination of VX on clothing material. The sampling and analysis was completed in less than 20.0 min, making this a relatively rapid detection method, especially considering the quality of the information provided. This method provides simplicity and an increased measure of safety for the field analyst since it does not require the handling of solvents for sample preparation. In addition, the analyst never directly handles the potentially contaminated sample, assuming samples are given to the analyst in containers suitable for SPME headspace sampling.

The SPME method investigated here may be seen as an interesting supplement to existing techniques that has potential utility in safely identifying unequivocal contamination. Additional work will be required to adapt the method for detection of trace level contamination. This is especially true for contamination of a complex medium such as that presented by soil.

ACKNOWLEDGEMENTS

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CHAPTER 4

DETECTION OF VX CONTAMINATION IN SOIL THROUGH SOLID PHASE MICROEXTRACTION SAMPLING AND GAS CHROMATOGRAPHY/MASS SPECTROMETRY OF THE VX DEGRADATION PRODUCT BIS(DIISOPR0PYLAMINOETHYL)DISULFIDE

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ABSTRACT

A solid phase microextraction (SPME) and gas chromatography/mass spectrometry (GC/MS) sampling and analysis method was developed for bis(diisopropylaminoethyl)disulfide (a degradation product of the nerve agent VX) in soil. A 30-minute sampling time with a polydimethylsiloxane-coated fiber and high temperature alkaline hydrolysis allowed detection with 1.0 μg of VX spiked per g of agricultural soil. The method was successfully used in the field with portable GC/MS instrumentation. This method is relatively rapid (less than

1 h), avoids the use of complex preparation steps, and enhances analyst safety through limited use of solvents and decontamination of the soil before sampling.

KEY WORDS: chemical warfare agents; VX; bis(diisopropylaminoethyl) disulfide; solid phase microextraction; gas chromatography/mass spectrometry; field analysis.

INTRODUCTION

The chemical warfare agent (CWA) O-ethyl S-(2-diisopropylaminoethyl) methylphosphonothiolate (VX) is an organophosphorus nerve agent. With a lowest lethal dose (LDLO) of 70 μg/kg [1], VX is arguably one of the most toxic CWAs, and its low vapor pressure allows it to be somewhat persistent in the environment. There is a need for rapid, reliable, and relatively simple field detection methods for persistent chemical warfare agents such as VX, when they exist as soil contaminants. An ideal field method will be rapid, safe for the analyst, and will provide orthogonal data, even at trace contamination levels, giving a high degree of certainty regarding analyte identity.

A number of chromatographic methods have been developed for identification of CWA's on soil using a variety of detectors [2-5]. Interest in

development of field sampling and analysis methods has grown in response to the demand for rapid field analysis in both the civilian and military communities [6-9] and fieldable gas chromatography/mass spectrometry (GC/MS) equipment is available. Using such instrumentation, data may be obtained that are of near equal quality to those produced in the laboratory, considering the instrument's sensitivity and usefulness of the resulting mass spectra. However, a major drawback to field GC/MS continues to be traditional sampling and sample preparation methods that require solvent extraction. Thermal desorption methods are available that bypass solvent use, but additional equipment and more complicated analysis procedures result when these are used, and they may not be easily adaptable to analysis of soil samples.

Solid phase microextraction has been used widely for environmental sampling and a thorough review of SPME background and methodologies is readily available [10,11]. SPME has been used for sampling and detection of CWA's in air and water [12-15]. SPME methods for detecting CWA's or their degradation products on soil have been developed for use with analytical methods such as GC/MS [16-18] and GC with flame photometric detection [18].

The usefulness of gas phase SPME coupled to GC/MS for field analysis of unknown chemicals in complex environmental matrices has been demonstrated [19]. Field sampling/analysis using gas phase SPME

with GC/MS analysis has included detection of CS riot control agent and thermal degradation products [20], and detection of VX as a clothing contaminant [21].

With a low vapor pressure, detection of VX on soil using a field analytical method that relies on the analyte being in the gas phase presents a challenge. Heating a sample that contains VX could volatilize sufficient analyte to allow headspace SPME sampling. However sample degradation issues argue against this approach for detecting the intact VX molecule. Bis(diisopropylaminoethyl)disulfide ($(DES)_2$, Figure 4-1) has been reported to be present in stored VX [22,23] and is an environmentally persistent degradation product of VX [24]. Small [24] reported $(DES)_2$ would be the likely surviving compound from VX contamination either after decomposition (without decontaminant) or from decontamination with a solution consisting of 70% diethylenetriamine, 28% methyl cellosolve, and 2% sodium hydroxide.

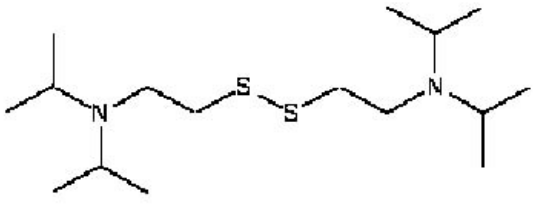


Figure 4-1. Bis(diisopropylaminoethyl)disulfide, $(DES)_2$

If this compound could be reliably produced from VX-contaminated material, and if it were stable enough to allow SPME sampling (possibly at elevated temperatures that would hasten its formation) it could serve as a useful marker for VX contamination.

This effort evaluates the use of headspace SPME with analysis by GC/MS as a relatively safe detection method for VX contamination on soil by identifying the presence of the degradation product $(DES)_2$ following high temperature alkaline hydrolysis of VX. In order to use SPME for detection of this analyte, sampling temperatures, fiber selectivity issues, and the kinetics of analyte loading onto the SPME fiber were studied. In addition to the study of these points, quantitative detection issues for $(DES)_2$ were evaluated in the laboratory using VX-spiked agricultural soil. Finally, the method was used in a field setting with VX-spiked soil. In addition to the potential usefulness of this method for soil with intact VX, it could also be useful in sampling for degraded VX in which $(DES)_2$ is already present.

From a safety perspective, the SPME methods discussed here avoid traditional solvent extraction, have a small logistical footprint, and sampling occurs from within a sealed system where the VX-contaminated soil has been at least partially decontaminated.

Materials and Methods

Materials

VX (95% purity) was obtained from the U.S. Army Edgewood Chemical Biological Center (Aberdeen Proving Grounds, MD). For the laboratory work, the VX was diluted in chloroform to 0.9 mg/mL and was handled at that concentration. VX (97% purity) was obtained from Defence Research and Development Canada - Suffield (Medicine Hat, Alberta, Canada) for the field studies. Standards for 2-(diisopropylamino)ethanethiol, bis(diisopropylaminoethyl)sulfide and $(DES)_2$ were synthesized. Bis(diisopropylaminoethyl)sulfide was produced by reacting 2-(diisopropylamino)ethyl chloride hydrochloride (Aldrich, Milwaukee, WI) with two equivalents of potassium thioacetate in acetonitrile. The resulting thioacetate was purified and reacted with ammonia in methanol to generate 2-(diisopropylamino)ethanethiol, which was coupled with 2-(diisopropylamino)ethyl chloride hydrochloride in acetonitrile with potassium carbonate. $(DES)_2$ was produced by exposing a sample of 2-(diisopropylamino)ethanethiol to atmospheric oxygen for 12 h, yielding the corresponding disulfide. The disulfide was distinguishable from the thiol and the sulfide by thin layer chromatography, ^1H NMR spectroscopy and GC/MS with 70ev electron impact (EI) and ammonia chemical ionization (CI) detection. An analytical standard for 2-(diisopropylamino)ethanethiol was obtained by adding $NaBH_4$ in

methanol to reduce $(DES)_2$ back to the thiol compound. For retention time and mass spectrum comparisons, liquid injections were made for each standard.

All SPME fibers and holders used in this study are commercially available from Supelco (Bellefonte, PA). The following five fiber coatings were evaluated: 100 μm polydimethylsiloxane (PDMS), 85 μm polyacrylate (PA), 65 μm carbowax/divinylbenzene (CW/DVB), 65 μm carboxen/polydimethylsiloxane (CAR/PDMS,), and 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB). Prior to use, each fiber was conditioned following the manufacturer's recommendations. Blank runs were completed a minimum of once daily before use of any fibers for sampling.

SPME Sampling

Selection of optimal fiber. Selection of the optimal SPME fiber for sampling $(DES)_2$ from among those tested was accomplished by obtaining triplicate samples from 15 mL silanized vials (without soil) having open screw top closures fitted with polytetrafluoroethane (PTFE)-lined silicone septa. Each vial was spiked with 2.0 μl of a standard solution (0.96 mg/mL $(DES)_2$) with a 100 μl syringe (Hamilton, Reno NV). To ensure reproducible

spiking, a solvent chase method was used in which 1.0 μL of chloroform was drawn into the syringe, followed by 1.0 μL of air, and then the measured aliquot of the $(DES)_2$ solution. The temperature of the vial sampled was maintained at 50 °C by placing the vial in a digitally controlled hot-block heater (Barnstead/Thermolyne, Dubuque, IA). Each sample was allowed to equilibrate in the hot-block for 10.0 min after which the septum was pierced with the SPME fiber assembly and the fiber extended into the vial for a 30.0 min extraction period.

At the end of the extraction period, the SPME fiber was retracted into its protective sheath, removed from the vial and immediately introduced into the heated GC injection port. The fiber was then lowered into the midrange region of the heated injection port liner (0.75 mm I.D. deactivated glass, Supelco) and GC/MS analysis commenced. The fiber providing the greatest GC/MS peak areas was selected for further sampling and analysis optimization.

Selection of Optimal Temperature and Sampling Time. Another set of spiked vials was analyzed using the optimal fiber to determine the effect of temperature on extraction. The extractions were performed under the same set of conditions used previously, except the temperature of extraction was varied (25, 50, 75, or 100 °C). Finally, the fiber was

exposed at the resulting optimal temperature selected over an increasing extraction time period to examine fiber uptake kinetics for $(DES)_2$.

Laboratory Soil Headspace SPME. Once the optimal extraction parameters from among those studied had been identified, SPME extraction of $(DES)_2$ from VX-spiked soil was completed. The soil used was Standard Reference Material (SRM) 2709, San Joaquin soil (National Institute of Standards and Technology, Gaithersburg MD). Soil samples were created by spiking 1.0 g SRM soil in silanized vials with 100 μL of VX solution (0.9 mg/mL) followed by mixing of the spiked soil within the vial using a vortex mixer for 30 s. Some of these soil samples were analyzed using headspace SPME at 50 °C and the method described by Hook *et. al.* [21] in an attempt to directly detect the presence of intact VX. To the remainder of the soil samples, 500 μL of decontamination solution (equal parts of 2.5 N NaOH and methanol) was added followed by an additional 30.0 s of mixing. These vials were placed in a heating block at 100 °C for a 10.0 min temperature equilibration period prior to the 30.0 min extractions. In order to estimate the sensitivity of the method for detecting $(DES)_2$, additional vials with soil and VX were prepared and sampled in this way. However, the mass of VX added to these vials ranged from 0.5 μg/g to 203.0 μg/g.

Soil Headspace SPME, Field Sampling/Analysis. Field samples were prepared by placing 1.0 g of the SRM soil in each of three silanized vials followed by spiking each vial with 90.0 μg of VX in a laboratory setting, and sealing each vial with a screw-top closure and PTFE-lined septum. Field analysis was performed the following day after applying 500 μl of decontamination solution to each vial. The 10.0 min temperature equilibration, 30.0 min extraction time, and 100 °C extraction parameters were used for these samples and all handling was completed in a portable fume hood equipped with an activated charcoal filtering system.

Statistical Analysis. Experimental data were examined for differences between $(DES)_2$ GC/MS peak areas. The statistical test used for this determination was the analysis of variance (ANOVA), which was completed for each of the three data sets. This was followed by Tukey's *post hoc* comparison to evaluate the source of observed differences. To examine reproducibility, the laboratory samples were run in triplicate and relative standard deviation (RSD) values were calculated.

GC/MS Methods.

The fiber optimization, temperature and extraction time samples for $(DES)_2$ were analyzed immediately following collection using a 6890 series

gas chromatograph and 5973 quadrapole mass selective detector (Agilent Technologies, Wilmington, DE). The GC was fitted with an Agilent, HP-5MS, 30m x 0.25 mm I.D. column having a film thickness of 0.25 µm. Helium at 1 mL/min was used as the carrier gas. The oven was programmed to increase from 40 to 250 °C at 20 °C per minute following a 2.00 min hold time at the initial temperature. Desorption of the SPME fiber samples was accomplished in the splitless injection mode for 2.00 min, followed by a 50 mL/min injector purge. The injector temperature was maintained at 250 °C throughout an analysis, and the mass spectrometer transfer line was kept at 270 °C. Electron impact ionization (GC/MS-EI) was used for most of these samples. Mass spectra were collected over the range of 35-350 m/z for GC/MS-EI, and chemical ionization (GC/MS-CI) analyses. GC/MS-CI operating conditions followed D'Agostino *et al.* [23] with anhydrous ammonia (99.99%, Aldrich) used as the CI reagent gas. Sample retention characteristics and mass spectra were stored using the Agilent Chemstation software package.

Due to VX handling constraints, laboratory SPME extraction samples of decontaminated VX on soil were analyzed using a different (but identically configured) GC/MS system with a J & W Scientific (Folsom, CA, USA) DB-5, 30m x 0.25 mm I.D. column having a film thickness of 0.25 µm. Operating parameters were as described above. Both GC/MS-EI and GC/MS-CI analyses were completed with this instrument.

GC/MS-CI with headspace SPME sampling of decontaminated VX provided molecular mass information for degradation products observed. Silanized vials were spiked with 45 μg of VX followed by application of 500 μl of decontamination solution. Extractions were performed using the same extraction conditions as before (100 °C, 10.0 min equilibration, 30.0 min extraction with PDMS fiber). GC/MS-CI was performed on the $(DES)_2$ and bis(diisopropylaminoethyl)sulfide standards by direct injection of dilute concentrations of each standard independently.

Field analyses were performed using a third GC/MS system (van mounted) of the same type used for laboratory samples with water electrolysis providing high purity H_2 carrier gas. This instrument was fitted with an HP-5MS column (30m x 0.25mm I.D., 0.25 μm film thickness) and operating parameters were identical to those used in laboratory GC/MS-EI analyses.

RESULTS AND DISCUSSION

Fiber Selection. Table 4-1 provides the data obtained from fiber selection experiments. The PDMS, PA, and CW/DVB fibers were found to provide the greatest sensitivity and statistically they provided peak area responses that were indistinguishable. The fiber of choice for further work was the PDMS fiber as it provided good sensitivity and it has already been

shown to be the optimal fiber for field sampling and analysis of intact VX [21].

Table 4-1. Optimal Fiber Selection, GC/MS Peak Area Counts for $(DES)_2$, 30 min extraction, 50 °C

Sample no.	PDMS	PA	CW/DVB	PDMS/DVB	CAR/PDMS
1	2.57×10^8	2.15×10^8	2.40×10^8	1.83×10^8	6.57×10^7
2	2.83×10^8	2.56×10^8	2.60×10^8	1.39×10^8	7.13×10^7
3	2.73×10^8	2.22×10^8	2.55×10^8	1.57×10^8	6.13×10^7
Mean	2.71×10^8	2.31×10^8	2.52×10^8	1.61×10^8	6.61×10^7
SD	1.29×10^7	2.19×10^7	1.05×10^7	2.17×10^7	5.00×10^6
RSD	4.77	9.47	4.17	13.48	7.57

Temperature and Sampling Time Selection. Table 4-2 provides the data resulting from temperature optimization experiments. Apparently due to its low volatility, $(DES)_2$ could not be detected at room temperature. The GC/MS peak area responses obtained at 50, 75 and 100 °C were not statistically different. For further work, 100 °C was selected for use to maximize the $(DES)_2$ production rate during the degradation of VX on soil.

Table 4-2. Optimal Temperature Selection, GC/MS Peak Area Counts for $(DES)_2$, 30.0 min. extraction, PDMS fiber

Sample no.	25 °C	50 °C	75 °C	100 °C
1	ND*	2.57×10^8	2.78×10^8	2.64×10^8

2	ND*	2.83×10^8	2.42×10^8	2.49×10^8
3	ND*	2.73×10^8	2.84×10^8	2.25×10^8
Mean	--	2.71×10^8	2.68×10^8	2.46×10^8
SD	--	1.29×10^7	2.31×10^7	1.94×10^7
RSD	--	4.77	8.60	7.90

*Non-detectable

Figure 4-2 presents the uptake curve obtained for $(DES)_2$. Statistically, the peak areas for the 20.0, 30.0, 45.0 and 60.0 min extractions were indistinguishable from each other yet different from the peak areas for 1.0 and 10.0 min.

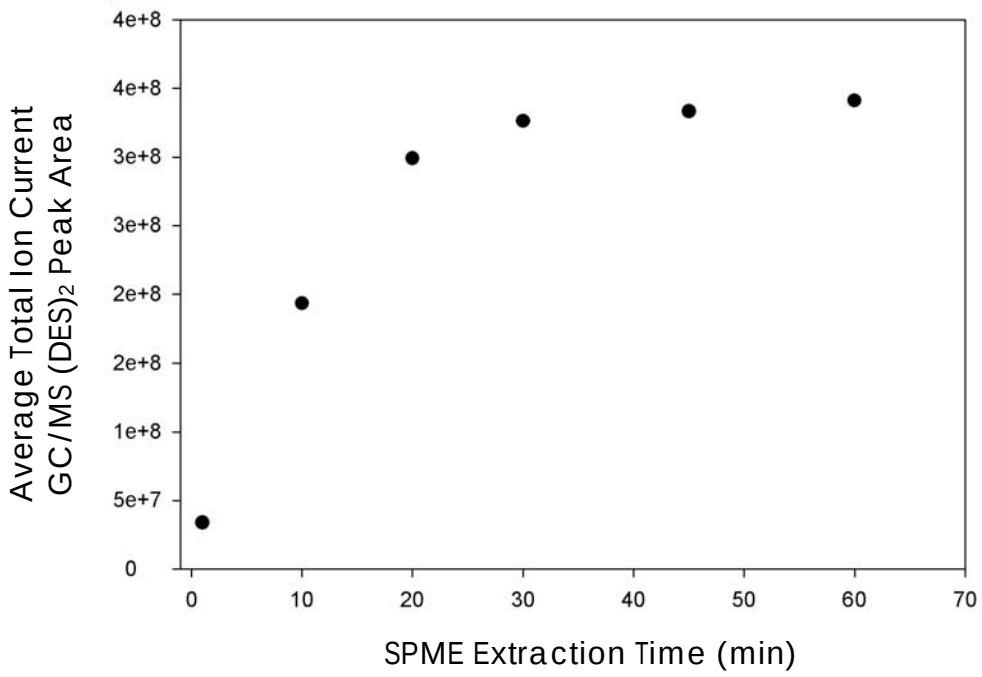


Figure 4-2. GC/MS-EI total ion current peak area for $(DES)_2$ plotted against SPME sampling time (PDMS fiber, 100 °C)

Compound Identification by GC/MS. Both GC/MS-CI and GC/MS-EI spectral and retention time matches were obtained for $(DES)_2$, 2-(diisopropylamino)ethanethiol, and bis(diisopropylaminoethyl)sulfide peaks using authentic standards. Figure 4-3 illustrates a GC/MS-CI total ion chromatogram from decontaminated VX. It is recognized that GC/MS-CI is not a method that would find use in typical field GC/MS analyses but its use here in the laboratory confirmed production of $(DES)_2$

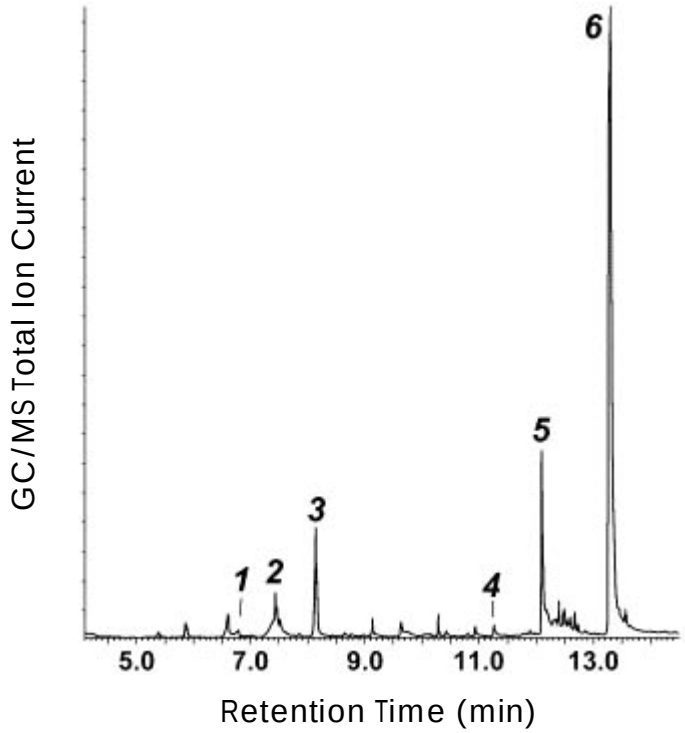


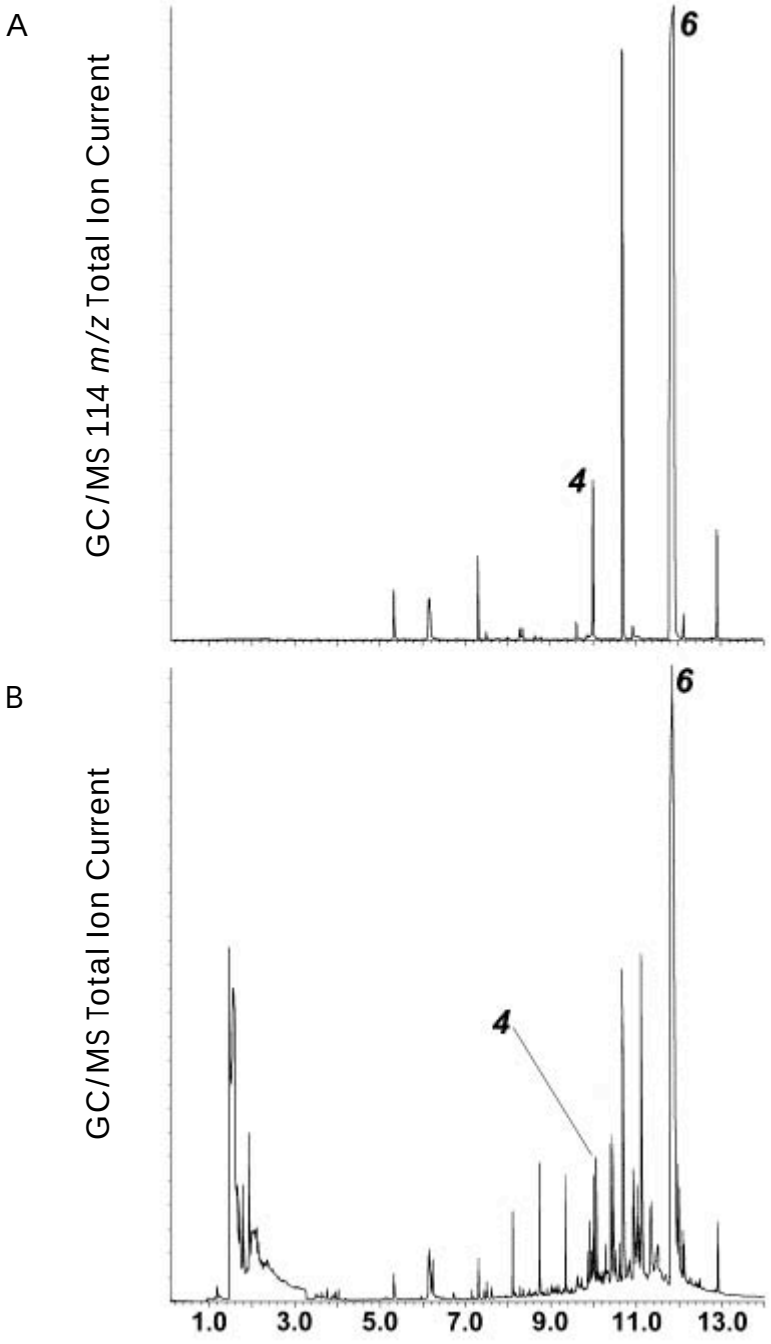
Figure 4-3. Ammonia GC/MS-CI chromatogram of VX subjected to alkaline hydrolysis; Compound key for Figures 3, 4a, 4b, and 6: 1 O, S,-diethylmethylphosphonothiolate[a], 2 2-(diisopropylamino)ethanethiol[b], 3 2(diisopropylamino) ethylmethyl sulfide[a], 4 VX[b], 5 bis(diisopropylaminoethyl)sulfide[b], 6 bis(diisopropylaminoethyl)disulfide[b]. [a]Identification based upon apparent CI pseudo-molecular ion only.

[b]Identification based upon apparent CI pseudo-molecular ion, retention time and EI spectrum match with authentic standard from alkaline hydrolysis of VX, and simplified interpretation of laboratory

generated data. The GC/MS-EI spectra of VX and its degradation products that contain the diisopropylaminoethyl functional group are dominated by the 114 *m/z* ion, and little unambiguous diagnostic information for these analytes is available from stable high mass ions. Degradation products for which standards were not available were thus identified based upon the pseudo-molecular and fragmentation ion data provided by GC/MS-CI, and by comparison to GC/MS-CI analyses performed by D'Agostino *et al.* [23]. In order to examine field samples for the presence of $(DES)_2$, authentic standards of the disulfide compound should be analyzed ahead of time to obtain a retention time for this analyte, a relatively easy procedure if the $(DES)_2$ standard is available.

Soil Headspace SPME. Initial studies of soil spiked with VX (no alkaline hydrolysis) demonstrated that SPME-GC/MS was unable to reproducibly detect the presence of intact VX at 50 °C. Additional attempts to detect intact VX at 100 °C were also unsuccessful. Field analysis for $(DES)_2$ from the decontamination of VX-spiked soil was successful and total ion and extracted 114 *m/z* ion chromatograms are

shown in Figure 4-4. $(DES)_2$ is the predominant peak, consistent with laboratory analyses. Formation of $(DES)_2$ from hydroxide-catalyzed



Retention Time (min)

Figure 4-4. (a) GC/MS-EI 114 *m/z* extracted ion trace from field analysis of 90.0 μg of VX spiked to SRM soil following alkaline hydrolysis, 10.0 min equilibration, and 30.0 in extraction with PDMS fiber at 100 °C; (b) Total ion chromatogram of same GC/MS data file as Figure 4a

degradation of VX has been observed previously [25]. This process is proposed to occur via the pathway given in Figure 4-5.

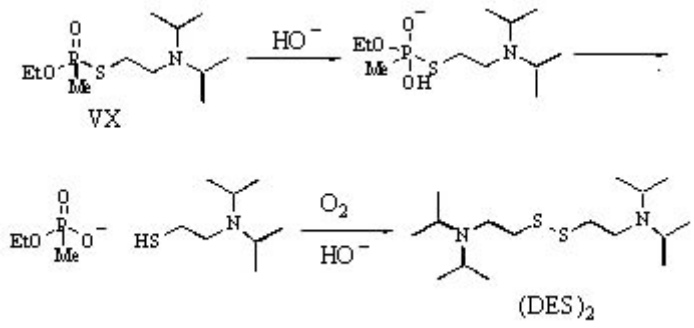


Figure 4-5. Mechanism for formation of $(DES)_2$ from hydroxide-catalyzed degradation of VX

At 100 °C with a 30.0 min extraction, $(DES)_2$ was detected with >3:1 signal-to-noise ratio down to a level of 1.0 μg of VX spiked to 1.0 g of the SRM soil (1 ppm). A linear response was observed from 1 to over 100 ppm VX soil concentration when plotting the logarithm of soil concentration against average GC/MS-EI $(DES)_2$ 114 *m/z* peak areas. A GC/MS-EI chromatogram (114 *m/z* extracted ion trace) is shown for a 1 ppm laboratory sample in Figure 4-6.

With the methods described here, $(DES)_2$ can be detected in soil from initially intact VX in less than one hour. It may be possible to reduce this time by eliminating the 10.0 min equilibration period, although we did not explore this possibility. This method may have application in detecting the presence of VX in complex media other than soil. As shown in Figure 4-4, the selection of the 114 *m/z* ion trace for detection of VX and its degradation products that contain the diisopropylaminoethyl functional

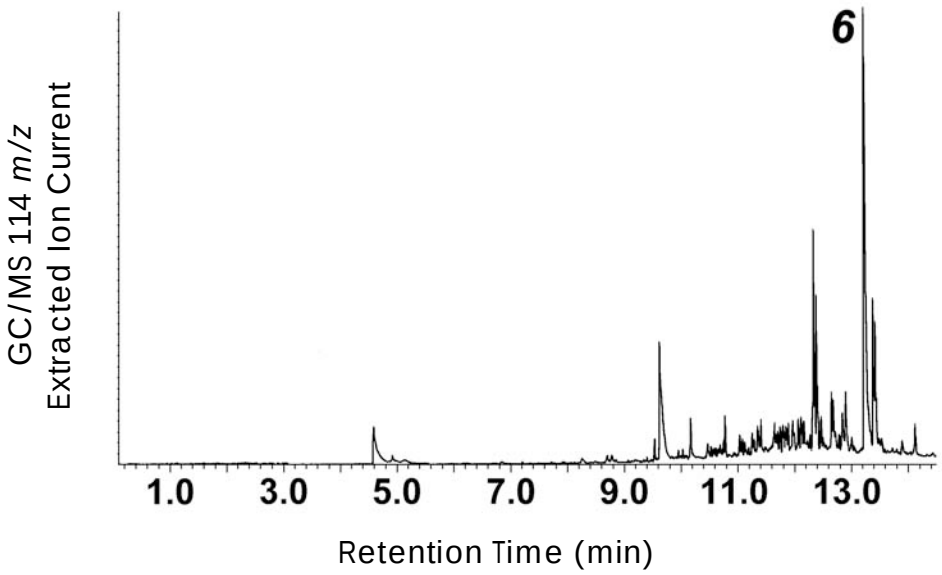


Figure 4-6. GC/MS 114 *m/z* extracted ion trace from analysis of 1 ppm VX spike on SRM soil following alkaline hydrolysis, 10.0 min equilibration, and 30.0 min extraction with PDMS fiber at 100 °C. $(DES)_2$ was observed in total ion chromatograms in all samples where the VX soil concentration was 15 ppm (m/m) or greater.

group enhances the field analyst's ability to identify compounds of interest in an otherwise complex chromatogram. Owing to the variables related to different soil types, quantitation of VX soil contamination would be difficult using the methods investigated here. However, based upon the orthogonal data produced by GC/MS-EI analysis, qualitative identification of $(DES)_2$ would be fairly unambiguous.

As a final word, analyst safety is important when using laboratory methods and instrumentation in the field to detect an analyte such as VX. This method promotes analyst safety by limiting the use of solvents to the small amount used for decontamination of the VX and generation of $(DES)_2$, and reduces exposure potential for the intact VX molecule. While the toxicity of $(DES)_2$ has not been well characterized in the literature, Munro *et. al.* [26] reported an estimated reference dose (RfD) for $(DES)_2$ as 6.6 μg/kg/day. This is 4 orders of magnitude higher than the RfD for VX [27] therefore, $(DES)_2$ is anticipated to be of much lower toxicity than VX.

CONCLUSION

Orthogonal data were provided using a field expedient SPME-GC/MS sampling and analysis method to detect the presence of VX soil contamination. With the overall desire to develop a simple field sampling and analysis method, sample preparation was limited to addition of a

small amount of alkaline methanol to silanized vials containing VX contaminated soil followed by heating at 100 ºC during the 30.0 min passive SPME headspace sampling time. Analyst safety is enhanced by the alkaline hydrolysis of VX in the soil sample and the intent to determine the presence of VX through the identification of the resulting VX degradation product $(DES)_2$. As completed here, the presence of VX on soil was detectable through the use of the $(DES)_2$ marker at concentrations as low as 1.0 $\mu g\ g^{-1}$ of soil (1 ppm, w/w). With a total sampling and analysis time of less than 1 h, high quality data for chemical identification is readily available. Even with the need for a heating block and decontamination solution, this method lends itself to field analysis as the complex sample preparation steps typically required for soil samples are avoided. In addition to enhanced analyst safety resulting from alkaline hydrolysis of VX in the sample, safety is further enhanced as SPME headspace sampling minimizes the potential for exposure to any contaminants present in the soil.

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CHAPTER 5

DYNAMIC SOLID PHASE MICROEXTRACTION FOR SAMPLING OF AIRBORNE SARIN WITH GAS CHROMATOGRAPHY – MASS SPECTROMETRY FOR RAPID FIELD DETECTION AND QUANTIFICATION

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ABSTRACT

A portable dynamic air sampler and solid phase microextraction were used to simultaneously detect, identify, and quantify airborne sarin with immediate analysis of samples using a field portable gas chromatography-mass spectrometry system. A mathematical model was used with knowledge of the mass of sarin trapped, linear air velocity past the exposed sampling fiber, and sample duration allowing calculation of concentration estimates. For organizations with proper field portable instrumentation, these methods are potentially useful for rapid onsite detection and quantification of high concern analytes, either through direct environmental sampling, or sampling of air collected in bags.

KEY WORDS: *sarin, solid phase microextraction, dynamic sampling, gas chromatography-mass spectrometry*

INTRODUCTION

The demand for field sampling and analysis for rapid determination of routine industrial, emergency response, and military related exposures to organic chemicals is increasing [1-5]. Colorimetric, infrared and ion mobility spectrometry methods have been used as rapid screening techniques for identification of unknown chemicals. False positive identifications are a potential with many of the current screening methods and follow-up confirmation of the results are often provided by an off-site laboratory.

GC-MS is widely acknowledged as a powerful method for detection and identification of unknown organic compounds. Relatively rapid qualitative analysis of industrial chemicals and chemical warfare agents (CWAs) in laboratory and field settings, using solid phase microextraction (SPME) coupled to gas chromatography-mass spectrometry (GC-MS), has been demonstrated [6-10]. However, a field friendly method providing near laboratory quality data for rapid quantitative sampling of airborne chemicals has remained elusive. Conventional methods for quantitative sampling of airborne chemicals

rely on capturing the analytes on a sorbent media. This typically requires the use of solvents and or additional equipment to desorb the analytes from the media in preparation for GC-MS analysis.

It is proposed that many of these limiting factors can be overcome through the use of a portable dynamic air sampler (PDAS) and solid phase microextraction (SPME) with both qualitative and quantitative analysis completed simultaneously using a field portable GC-MS system. SPME, first described in 1990 [11], is now a well-established method with its background and methodologies thoroughly documented [12,13]. SPME has been widely applied in laboratory and environmental settings. In particular, environmental SPME sampling has been useful for a variety of industrial chemicals, pesticides, and CWAs in air, water and soil [6,7,10,12-16]. Schneider *et al.* [17] demonstrated the usefulness of SPME as a rapid response screening tool for detection of isopropyl methylphosphonofluoridate (sarin) in air and water. In that work, samples were obtained in the field and returned to the laboratory for analysis. Harvey *et al.* [18] demonstrated improved selectivity for sarin through use of SPME with a phenol-based polymer coating as opposed to a commercially available fiber coating. Usually SPME, samples are collected using passive sampling.

Koziel *et al.* [19] and Augusto *et al.* [20] have demonstrated the capability of dynamic SPME sampling with a PDAS device. PDAS-SPME

was effective for rapid quantitative analysis of a variety of air contaminants. In their work, quantification with the PDAS device was obtained mathematically without the need for gas-phase standards and demonstrated the method's potential for field use. Furthermore, their work showed PDAS-SPME outperformed static SPME sampling in terms of method sensitivity and precision.

The basis for PDAS-SPME is to draw the air being sampled perpendicularly across a SPME fiber coated with a mixed porous solid adsorptive phase at a known, constant rate. Koziel [19] established 10 cm s^{-1} as the critical linear sampling velocity at which the mass uptake rate of the fiber becomes nearly constant at a fixed analyte concentration, and is controlled by the diffusion of the analyte through the boundary layer between the bulk air and the surface coating of the adsorbtive SPME fiber used for sampling.

A field-friendly method that provides rapid identification and quantification of highly dangerous airborne chemicals is of obvious benefit to both military and civilian populations. While the use of nerve agents in warfare has been limited, sarin has been used twice in Japan in acts of terrorism resulting in a total of 19 deaths and numerous casualties [21]. Sarin is a volatile G type nerve agent and is highly toxic with an estimated human lethal concentration (LCt_{50}) of 100 mg•min m^{-3} [22]. The goal of the work described here was the development of a rapid, field

friendly method for identification and quantification of airborne sarin using PDAS-SPME, followed immediately by GC-MS analysis using a field portable instrument.

MATERIALS AND METHODS

Safety Considerations

As sarin is known to be a dangerous compound, numerous safety precautions were taken to complete this work. These included the availability of standard military defensive medications, and a properly fitted military gas mask for each researcher located in the laboratory where the work was performed. Laboratory gloves and clothing known to be effective against this compound were also worn. All chemical handling was completed under a hood equipped with exhaust that was continuously scrubbed, and chemical handling protocols were followed that limited the potential for exposure.

Materials

Sarin (>95% purity) was obtained from Defence Research and Development Canada – Suffield (Medicine Hat, Alberta, Canada). The SPME fiber with a 65 μm polydimethylsiloxane/divinylbenzene (PDMS/DVB) coating (Supelco, Bellefonte, PA) was used for this work based upon previous fiber optimization studies where this coating gave much greater

GC-MS peak areas compared to the other commercially available adsorptive fiber coating (carboxen/PDMS). Each fiber used was conditioned following the manufacturer's recommendations prior to use. Blank runs were completed a minimum of once daily before use of any fibers for sampling.

Air Sample Generation

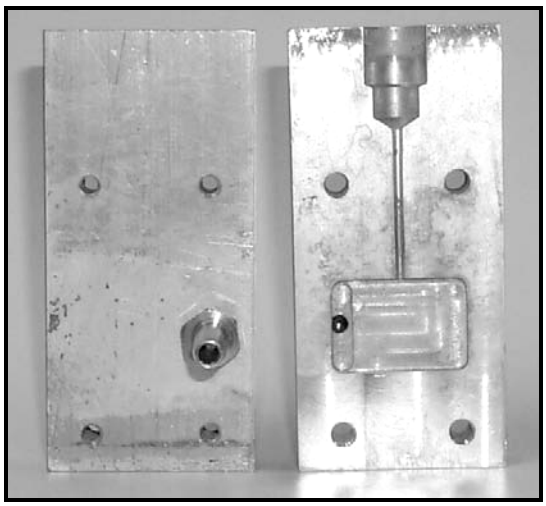
Static air samples in polytetrafluoroethylene (PTFE) bags (SKC Inc, Eighty Four PA) were generated at four concentrations ranging from 0.1 mg m^{-3} to 5.0 mg m^{-3}. A 1.0 L syringe (Hamilton, Reno, NV) was used to deliver 4.0 L of clean air to each bag. Standard dilutions of neat sarin were made in methylene chloride (99+%, Aldrich, Milwakee WI) and subsequently injected into the bags through a PTFE-lined silicone septum present in each bag, to achieve the desired concentrations following sarin evaporation. All chemical handling was performed in a fume hood where hood effluent was scrubbed through a charcoal filter.

Static SPME and Dynamic-SPME Air Sampling

Prior to performing the dynamic sampling, static samples were obtained directly from the PTFE bags using the PDMS/DVB fiber. These samples were performed in triplicate with 60.0 s sample duration for each extraction. Dynamic sampling was performed using a PDAS based upon

the concept originally reported by Koziel *et al.* [19] and later by Augusto *et al*. [20]. The PDAS used is shown in Figure 5-1. For safety reasons the PDAS developed here was designed with a fitting on the inlet to allow dynamic sampling from an air sampling bag. This design does not preclude the use of this unit for direct sampling of a potentially contaminated environment.

5-1a



5-1b

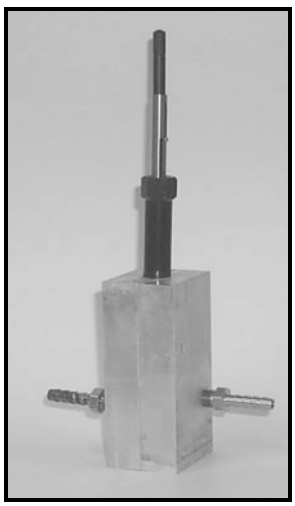


Figure 5-1. (a) PDAS device for SPME sampling showing exterior (left) and interior (right) views. The external PDAS dimensions are 10.2 cm (H) x 4.9 cm (W) x 3.7 cm (D). Dimensions of internal sampling zone (both halves combined to make complete sampler) are 2.1 cm (H) x 3.2 cm (W) x 0.80 cm (D). The sampling area is identical on both halves of the PDAS. (b) PDAS device with SPME fiber inserted.

The PDAS device was precision machined from aluminum stock that allowed the device to seal tightly without the need for gasket material or vacuum grease. The top of the PDAS device was recessed to securely

hold the SPME fiber assembly during sampling. Immediately below this recess was an 11 mm PFTE/silicone septum (Supelco) that was held in place by machining the recessed area where it sits to be slightly smaller than the diameter of the septum. The SPME fiber pierced the septum in order to reach the sampling zone. A pump drew in air from the front of the sampler and through the sampling zone and the effluent exited from the back of the sampler on the end opposite the inlet before passing to the pump.

Through use of the air sampling pump (Sensidyne, Clearwater, FL) operating at an average flow rate of 2.16 lpm, an average linear sampling velocity of 21 cm s^{-1} was obtained. This was in excess of the 10 cm s^{-1} threshold sampling velocity [19] that is needed to ensure the mass uptake rate remains nearly constant. The excess velocity also ensured that minor variation inherent in the type of sampling pump used did not impact the mass uptake. The pump was calibrated with a Bios Dry Cal device (Bios International, Butler, NJ) before and after samples were collected at each of the four concentrations.

PTFE tubing ran from the air-sampling bag to the PDAS inlet, and from the PDAS outlet to the air-sampling pump inlet. This allowed air to be drawn from the bag and across the SPME fiber in the PDAS. Air exiting the sampling pump outlet was sent to a scrubber containing a methanol/potassium hydroxide decontamination solution. The entire

system was maintained within the chemical handling hood described previously.

Duplicate 30.0 s PDAS samples were collected on a PDMS/DVB fiber for each concentration. Triplicate samples were desired; however, safety concerns limited the volume of sarin-contaminated air that could be handled during each phase of the work and therefore limited the number of replicate samples for each concentration. Upon completion of each sample, the fiber was retracted into its protective sheath and immediately subjected to GC-MS analysis.

GC-MS Methods

For sample analysis, the SPME fiber samples were desorbed thermally in the injection port of a portable Viking Spectra Trak 572 GC-MS system (Bruker Daltonics, Billerica MA). The MS section of this instrument is based on a Hewlett Packard 5972 ion source and monolithic quadrupole mass filter. The Viking instrument measures (L x W x H) 61 cm x 31 cm x 45 cm. Its weight, without an external gas source or roughing pump is approximately 35 kg. 110 V (AC) power is required for its operation (about 400 watts under typical operating conditions). The size and power requirements of the instrument allow for field-portability, and operation from within a vehicle or structure near the scene of sampling.

The injection port, as used for SPME samples, was equipped with a deactivated injection port liner designed for thermal desorption of analytes from a SPME fiber (0.75 mm I.D., Supelco). All sample analyses were performed using a 30 m x 0.250 mm I.D. DB1-MS column (0.25 μm film thickness, J&W Scientific, Folsom CA) with He carrier gas and an initial linear velocity of 45 cm/s. Temperatures were: 175 °C (injection port and transfer line), 175 °C (MS transfer line), and 170 °C (MS ion source). GC oven temperature began at 35 °C, was held there for 1.0 min and then increased at 20 °C/min to 150 °C. EI (70eV) ionization was used and mass spectra were collected over 10 – 250 mass-to-charge ratio (*m/z*) range. Splitless injection was used with 50 mL min^{-1} split flow started at 2.0 min into each GC-MS run. The instrument split vent was vented into the fume hood with exhaust passed through charcoal scrubbers.

Quantitative Analysis of Liquid Standards

In order to estimate the mass of sarin loaded onto a SPME fiber, a MS detector response calibration curve was generated from liquid injections of sarin standards. The curve was determined by plotting the mass of sarin injected in a liquid sample against the 99 *m/z* extracted ion sarin peak area for four concentrations, with the mass of sarin injected ranging from 24 to 325 ng. All liquid injections completed during this work involved a solvent plug method. First, enough neat solvent was drawn into

the syringe to fill the needle, with 0.5 μL additional visible in the syringe barrel. Following this, 1.0 μL of air was pulled into the syringe followed by the amount of liquid mixed with sarin to be injected (verified by sight). This was followed by pulling 1.0 μL of air into the needle, and then just enough solvent to fill the needle. The result was a known volume of sarin dissolved in methylene chloride, with two air plugs on either side, and with solvent both in front of and behind the air plug nearest and furthest from the needle tip respectively. This ensured that the sarin dissolved in methylene chloride was delivered into the injector. The same instrument and conditions used to analyze the SPME samples were used to analyze the liquid injections, with the exception that the appropriate split/splitless or SPME injection liner was installed depending on the analysis.

Quantification of the PDAS-SPME samples was performed using relationships previously described [19,20] where the concentration in the gaseous matrix, C_g (ng mL^{-1}), can be determined using equation (1).

$$C_g = n \ln [(b + \delta)/b]/2\pi D_g L t \quad (1)$$

In equation (1), the amount of analyte extracted n (ng), is determined from the peak area and the detector response factor or calibration curve. Other variables in calculating the analyte concentration in the air sampled are the fiber radius b (cm), the thickness

of the fiber's effective static boundary layer δ (cm), the analyte diffusion coefficient in air D_g (cm^2 s^{-1}), the fiber length L (cm), and the sampling time t (s).

D_g can be obtained from literature as was done for this work or it can be estimated using the Fuller-Schettler-Giddings model [19,20]. δ can be determined using equation (2) [19,20] in which Re is the Reynolds number described in equation (3) and Sc is the Schmidt number described in equation (4).

$$\delta = 9.52b/Re^{0.62}Sc^{0.38} \quad (2)$$

$$Re = 2u/bv \quad (3)$$

$$Sc = v/D_g \quad (4)$$

Variables in equations 3 and 4 include the linear sampling velocity (u, cm s^{-1}) and the kinematic viscosity of air (v, cm^2 s^{-1}) which can be obtained from literature [23].

Results and Discussion

The design of the PDAS used in this work provides flexibility in obtaining dynamic samples. The sampler can be used to obtain samples

from an air sampling bag, as was done in this work, provided the volume of air in the bag is sufficient for the sampling velocity and sample duration employed. This can be of benefit when safety and/or security concerns limit access to potentially contaminated areas as could be the case in emergency response scenarios. In these situations, multiple air sampling bags can be filled in the contaminated zone and removed to an adjacent clean area for dynamic sampling and analysis. The PDAS device can also be used to sample directly from the contaminated environment as seen in previous work [20]. While this work was completed entirely in a laboratory setting for safety reasons, it demonstrates that PDAS sampling with analysis on a field portable GC-MS instrument has the potential to provide useful qualitative and quantitative data rapidly.

Figure 5-2 illustrates the sensitivity and high quality of data available with this rapid PDAS-SPME system. The sarin peak was near 3:1 signal-to-noise for detection in the total ion chromatogram of the 0.10 and 0.20 mg m^{-3} concentrations, however, it was readily detected at 2.5 and 5.0 mg m^{-3}. With a 30.0 s sample, the analyst can readily detect and quantify the presence of sarin at half of the immediately dangerous to life and health (IDLH) value (0.20 mg m^{-3} is the IDLH value) by viewing and integrating the 99 *m/z* extracted ion chromatogram. In more concentrated samples, PDAS-SPME allowed identification of sarin by mass spectrum match from the obvious total ion GC-MS sarin peak and simultaneous quantitation

using equation (1). Figure 5-3 shows a 99 *m/z* extracted ion chromatogram for a 30.0 s PDAS-SPME sample of sarin at 0.10 mg m $^{-3}$ concentration.

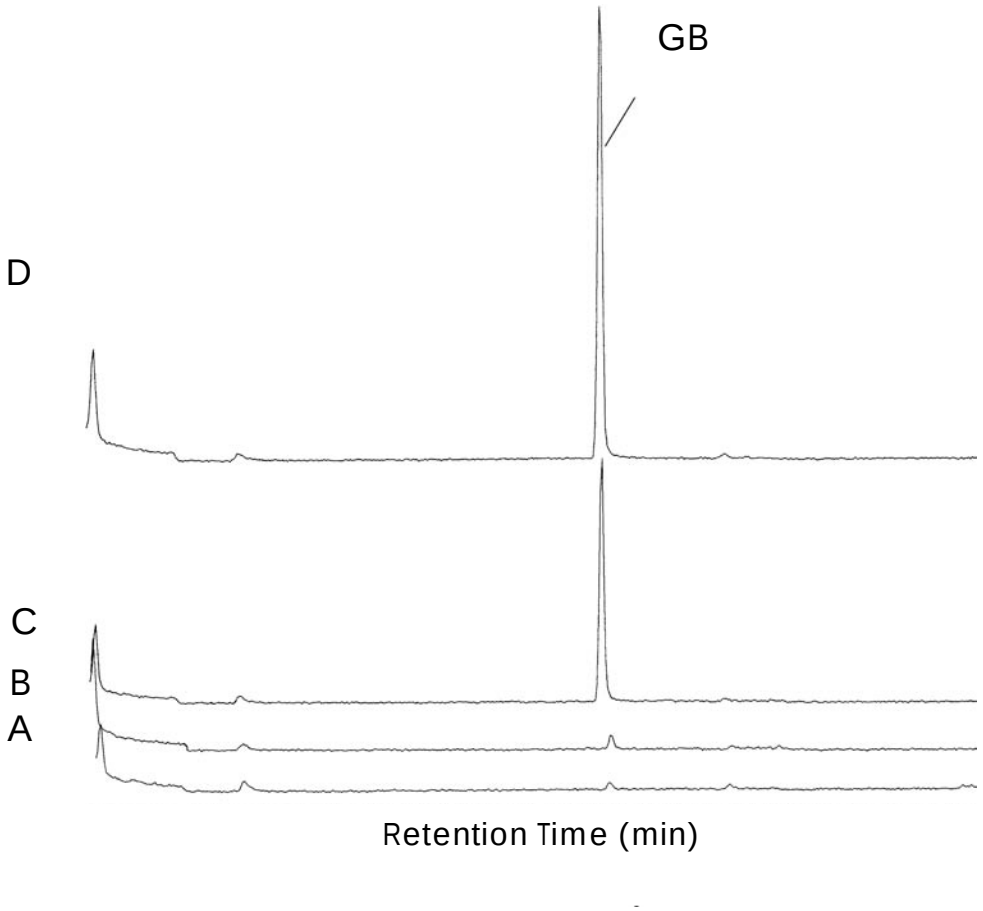


Figure 5-2. GC/MS chromatograms resulting from dynamic SPME samples of sarin at concentrations of A 0.10, B 0.20, C 2.5 and D 5.0 mg m^{-3}. The mass of sarin loaded on the fiber represented by the peaks was 13.9, 15.9, 73.4, and 129.1 ng respectively.

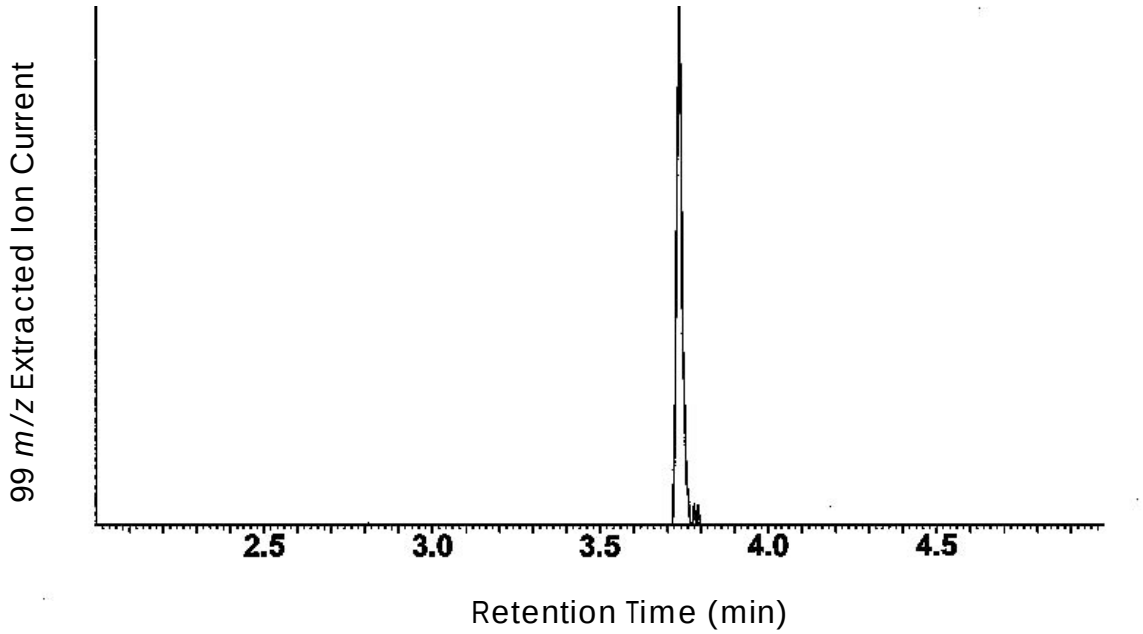


Figure 5-3. 99 *m/z* extracted ion trace from 30.0 s dynamic SPME sample of sarin at 0.10 mg m^{-3} concentration.

The calibration curve generated from liquid injections of sarin standards provided a linear response with a linear correlation coefficient (r^2) value of 0.985. From this curve, the mass of sarin loaded on the fiber (*n*) during each sample was determined and used to estimate the airborne concentration using equation (1). By performing periodic calibration curves, a PDAS-SPME-GC-MS system can be ready for quantitative analysis as the need arises without generating a gas phase calibration curve as would be needed for quantitative passive SPME sampling. The PDAS-SPME samples provided about equal sensitivity at half the sampling time compared to their corresponding static samples as

shown in Table 5-1. A linear response was achieved for both the PDAS-SPME and static SPME samples with r^2 values of 0.999 and 0.997 respectively when plotting the sarin concentration in the PTFE sampling bag against the resulting GC-MSpeak areas (99 *m/z* extracted ion current).

Table 5-1. GC/MS Peak Area Counts for Sarin Following PDAS-SPME and Static-SPME Extractions

Concentration	[1]PDAS-SPME	[2]Static-SPME
0.1 mg/m^3	2.64 x 10^4	2.55 x 10^4
	2.70 x 10^4	2.76 x 10^4
		2.07 x 10^4
0.2 mg/m^3	5.27 x 10^4	5.49 x 10^4
	5.42 x 10^4	4.46 x 10^4
		5.11 x 10^4
2.5 mg/m^3	8.53 x 10^5	8.12 x 10^5
	8.36 x 10^5	7.45 x 10^5
		8.10 x 10^5
5.0 mg/m^3	1.62 x 10^6	1.32 x 10^6
	1.60 x 10^6	1.45 x 10^6
		1.59 x 10^6

[1] 30.0 s sample duration
[2] 60.0 s sample duration

When estimating the thickness of the boundary layer, Augusto [20] substituted the threshold value 10 cm s^{-1} for the actual linear sampling velocity since velocities in excess of the threshold were assumed to not have a significant effect on the boundary layer thickness. Figure 4

demonstrates that substitution of the threshold value appears to give a better estimate of the actual concentration. The slope of the lines reveals this also. The perfect system would generate a line with a slope of 1. The line represented by the use of threshold velocity to estimate δ in figure 5-4 had a slope of 0.90, whereas the line generated by estimating δ using the actual sampling velocity had a slope of 0.60. The threshold calculation

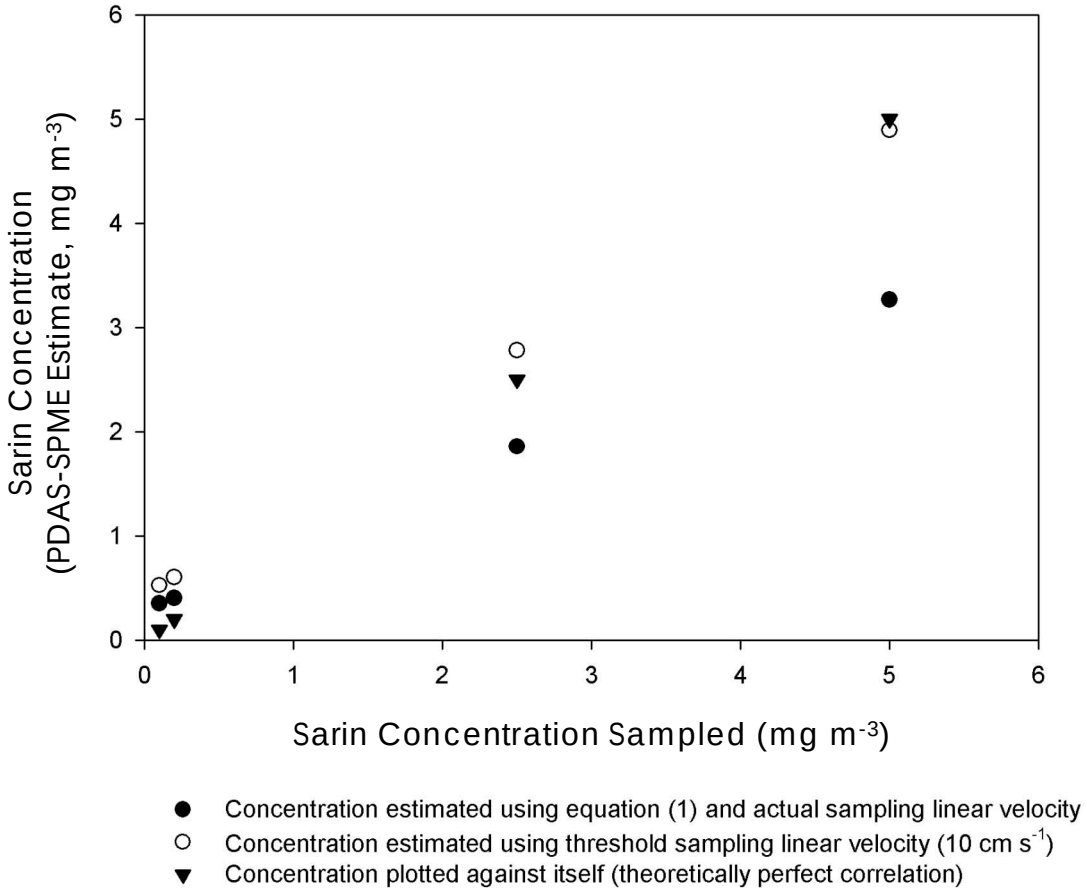


Figure 5-4. Comparison of dynamic SPME sample concentration estimates using actual and threshold sampling velocities.

method provided a more accurate estimate for higher concentrations. Both methods overestimated the concentration at the lowest two points. Over estimation of the lower concentrations, while not ideal, is preferable to underestimation when determining the level of protective equipment to be used in response to a sarin incident.

It should be noted that sampling for short periods of time (<1 min) is advisable to limit the potential problems associated with competetion for adsorbtion sites from other matrix components. In their work, Koziel et al [19] showed that at relative humidity levels near 50 % and for short PDAS sampling times (<1 min), uptake of benzene was not significantly affected compared to sampling the same concentration with 0% relative humidity.

Koziel et al. also recommend selection of appropriate sampling time "by collecting and analyzing several air samples and observing the linearity of the extraction curve" [19]. A rapid GC-MS analysis would be useful to accomplish this, and the ability to complete rapid analyses of CWA compounds by GC-MS using field-portable instrumentation has been demonstrated recently by Smith et al. Analysis of 4 volatile chemical warfare agents (including sarin) was completed by GC-MS in less than 2 min using a typical 30 m bonded liquid phase GC column similar to that used in this work [24]. In that work, the column was configured as a low thermal mass assembly, with resistive heating, and using high velocity H_2 carrier gas generated electrolytically on-site.

CONCLUSION

Barriers to the use of field portable GC-MS instruments for quantitative analysis of dangerous chemicals include the need to complete solvent extraction of air sampling media, or the need to use additional hardware (in the case of thermal desorption). These barriers are lessened by the use of PDAS-SPME-GC-MS, as only simple equipment is needed for sampling, and no additional equipment is needed to introduce the sample into a GC-MS system with a typical injector. The PDAS-SPME-GC-MS system described here thus provides a rapid method for detection, identification, and quantitation for sarin, and potentially for other airborne chemicals under field conditions. Dynamic air sampling followed by GC-MS analysis for sarin was completed in less than 10 min. However, a highly trained analyst is still needed to interpret the GC-MS data obtained, and chemicals likely to be encountered must be anticipated in order to complete liquid injection calibration curves beforehand, allowing a rapid response to an emergency situation.

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Chapter 6

CONCLUSION

DISCUSSION OF RESEARCH FINDINGS

In chapter 2, applied examples of the use of SPME with GC/MS analysis in dramatically different field scenarios were provided. The sampling and analysis of the poorly characterized Formula 150 paint demonstrated SPME is a powerful tool for routine evaluation of industrial environments. With just a 10 min sampling time and on-scene GC/MS analysis, highly reliable data demonstrating the presence of numerous substituted benzene compounds were obtained. These data provide a more thorough characterization of the paint components present as air contaminants than was provided by the manufacturer on the MSDS and by years of industrial hygiene monitoring.

Although not part of the original research plan, the opportunity to utilize SPME with GC/MS in an emergency response scenario was provided by the tragic events of September 11, 2001. The portability and simplicity of SPME sampling proved to be of benefit when obtaining samples in the totally uncharacterized environment presented by the Pentagon crash site. In both of these SPME sampling applications with GC/MS analysis in the field, the important question of: "What chemicals are present?" was

rapidly answered with high quality data. In the case of the crash scene effort, this directed and allowed meaningful quantitative analyses to follow.

Conditions for sampling VX were optimized in chapter 3. Here it was determined VX sensitivity is greatest when sampling with a PDMS fiber while the sample is maintained at 50 °C. Under these conditions, SPME-GC/MS was completed in less than 20 minutes. The method was shown to be a viable field sampling and analysis method for detecting gross (approximately 1 mg) VX contamination on clothing. The clothing material presented a simple medium from which to sample the VX. However, sampling VX in a more complex medium such as soil presents added difficulty, which was the focus of chapter 4.

Detecting the presence of VX on soil presents significant difficulty for rapid field methods. Chapter 4 provided evidence that VX contamination in a complex organic medium can be detected in the field by identifying the presence of bis(diisopropylaminoethyl) disulfide $(DES)_2$. This compound is a stable VX degradation product that results from either naturally occurring environmental hydrolysis or from alkaline hydrolysis associated with decontamination. Analyst safety is enhanced with this method since a potentially contaminated sample is decontaminated with the intent of generating $(DES)_2$ as an indicator of the VX contamination. The SPME-GC/MS method developed identified

the presence of VX contamination in soil using the $(DES)_2$ marker with sampling and analysis being completed in less than 1 hour for concentrations as low as 1.0 $\mu g\ g^{-1}$ of soil (1 ppm, w/w).

The ability to answer the question: "What chemicals are present?" in a relatively rapid manner with sensitive and reliable data has been demonstrated in the previous chapters. Chapters 2 through 4 utilized passive SPME sampling to obtain samples for anlaysis. Chapter 5 focused on the use of dynamic SPME sampling with GC/MS analysis in order to obtain data for a simultaneous qualitative and quantitative analysis. Through the use of 30.0 s dynamic SPME sampling, airborne sarin was detected and quantified at concentrations down to half the IDLH concentration (0.1 mg m^{-3}). As designed, the method provides a means for obtaining quantitative samples directly from the potentially contaminated environment or from air samples taken from the contaminated environment with an air sampling bag.

As demonstrated, SPME coupled to GC/MS provides a viable means for both qualitative and quantitative field sampling and analysis. It has application in a wide range of scenarios. For well characterized industrial workcenters, SPME-GC/MS can provide a rapid and sensitive means for confirming the adequacy of MSDS data in regards to the chemical constituents of compounds in use. It can be further used to rapidly characterize new materials as they are brought in to workcenters.

SPME-GC/MS provides emergency responders and the military with the capability of rapidly obtaining near laboratory quality data in the field. This is the beginning of the process of characterizing the unknown environments faced in operational environments with follow-on quantitative work impossible without first making a qualitative identification. This work shows SPME-GC/MS has potential for helping the military meet its requirement to evaluate and document troop deployment exposures.

The orthogonal data provided by the GC/MS significantly reduces the potential for false positive identification that is common to many other types of field detection equipment. However, this benefit does come with a price. The most significant price for rapid, high quality data is a well-trained and experienced analyst. Relying exclusively on the compound identification results provided by the GC/MS data handling computer is a poor approach. The well-trained and experienced analyst can interpret the data and reduce the potential for errors.

RECOMMENDATIONS FOR FUTURE RESEARCH

Further research regarding rapid field sampling and analysis methods providing simultaneous qualitative and quantitative capabilities is in high demand. Additional effort is warranted for optimization of SPME

sampling methods for other toxic industrial chemicals that are of high concern as well as the remaining CWAs. These efforts should focus on SPME sampling from various environmental matrices including air, water and soil. Furthermore, additional stationary phases should be explored in an effort to identify SPME coatings that have a greater affinity for the chemicals of concern and therefore provide greater sensitity.

Methods developed in further work should not only be developed for analysis with GC/MS systems of the types utilized in this work but also for additional analytical instruments such as the ion mobility spectrometry (IMS). GC/MS technology is continually evolving; therefore, research efforts should not be limited to development of sampling methods for field use. For example, low thermal mass, resistively heated columns for more rapid GC separations are available and should be evaluated concerning their potential for use in field portable systems.